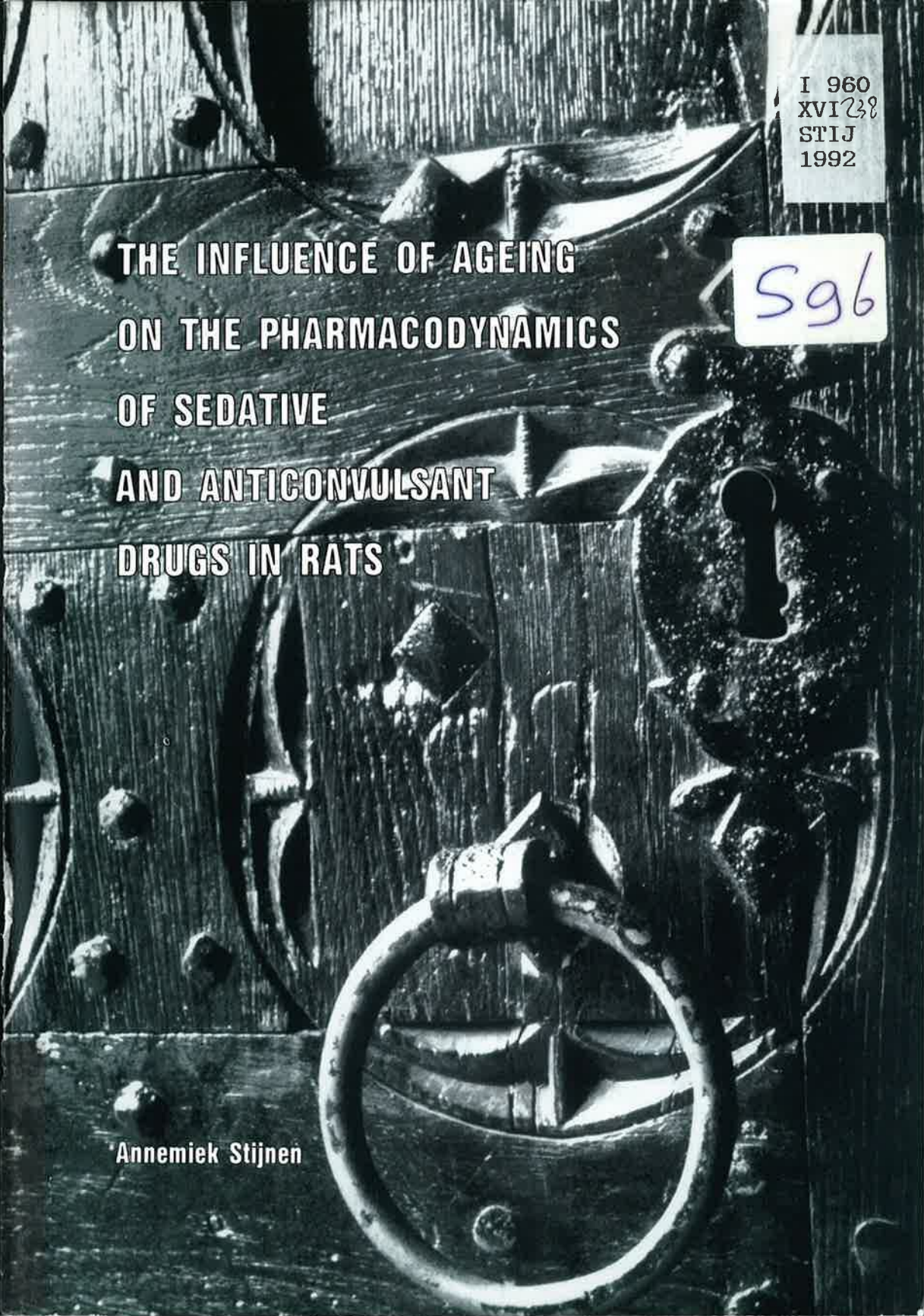

THE INFLUENCE OF AGEING ON THE PHARMACODYNAMICS OF SEDATIVE AND ANTICONVULSANT DRUGS IN RATS

Annemiek Stijnen

THE INFLUENCE OF AGEING ON THE PHARMACODYNAMICS OF SEDATIVE AND ANTICONVULSANT DRUGS IN RATS

STELLINGEN

1. De struktuur van de door Dingemanse et al. gerapporteerde metaboliet van heptabarbital geeft aanleiding te stellen, dat indirekte methodes om de farmacologische activiteit van één metaboliet vast te stellen hiertoe niet goed bruikbaar zijn.
   (Dingemanse et al., J. Pharm. Pharmacol. 40: 552-557, 1988; Heeremans et al., J. Chromatogr. 554: 205-214, 1991)

2. Voor een betrouwbare bepaling van de gezondheidstoestand van proefdieren is naast het verzamelen van klinisch biochemische gegevens een uitgebreid *post mortem* weefselonderzoek vereist.
   (Dit proefschrift)

3. Om leeftijdsgerelateerde veranderingen in de farmacodynamiek van stoffen met een werking op het centraal zenuwstelsel te kunnen detecteren is *in vitro* onderzoek ontoereikend, omdat homeostatische mechanismen, die een rol spelen bij de totstandkoming van het farmacologisch effekt, hiermee onvoldoende kunnen worden onderzocht.
   (Dit proefschrift)

4. Het verbeteren van statistiekonderwijs voor wetenschappelijke onderzoekers zou een significante verbetering kunnen betekenen voor de kwaliteit van wetenschappelijke publicaties.
   (Godfrey, N. Engl. J. Med. 313: 1450-1456, 1985)

5. De 'pseudo'-longitudinale onderzoeksopzet, zoals beschreven in dit proefschrift, is een belangrijk alternatief voor de cross-sectionele en de gewone longitudinale opzet.

6. De invloed van ziekte op de resultaten van dierexperimenteel verouderingsonderzoek wordt onderschat.

7. Om een verantwoorde geneesmiddeltherapie bij ouderen mogelijk te maken is farmacokinetisch en farmacodynamisch onderzoek volgens de populatiebenadering nodig, gezien het heterogene karakter van deze belangrijke doelgroep van farmacotherapie.

8. Voor een goede interpretatie van resultaten van verouderingsstudies in een diermodel zijn gegevens over de overlevingskarakteristieken van de dieren onontbeerlijk.

9. Ondanks het emancipatiebeleid van de Leidse Universiteit is er geen goed alternatief voor de 'Centrum voor Bio-Farmaceutische Wetenschappen-stropdas' als geschenk voor vrouwelijke gasten.

10. Mensen met afwijkende ideeën zouden vaker gezien moeten worden als bron van inspiratie dan als bron van irritatie.

11. Voor een juiste interpretatie van de dienstregelingen op NS-stations is het van belang om te weten dat een etmaal loopt van 04.00 u tot 04.00 u.

12. De discrepantie tussen de eisen die wat betreft onderwijsbevoegdheid gesteld worden aan docenten middelbaar onderwijs en universitaire docenten suggereert ten onrechte een vanzelfsprekende didactische bekwaamheid van de laatstgenoemde groep.

13. Het plaatsen van plastic bloemen en planten in Nederlandse openbare gebouwen en horeca-gelegenheden is een blamage voor een land dat een aanzienlijk deel van zijn internationale faam ontleent aan de bloementeelt.

14. Het onderzoek beschreven in dit proefschrift is een duidelijk voorbeeld van de meerwaarde van multidisciplinair onderzoek.

15. Gezien de hoge leeftijd van de alombekende heilige Nicolaas, is het berijden van een schimmel wellicht een gezonde sport voor ouderen.

16. De algemene bezorgdheid over het verschijnen van grijze haren is onterecht.
(Spreuken 20 vers 29)

17. Wie niet met carnaval in het bloed geboren is, zal het nooit in hart en nieren kunnen vieren.

Annemiek Stijnen

Leiden, 12 december 1991

THE INFLUENCE OF AGEING ON THE PHARMACODYNAMICS OF SEDATIVE AND ANTICONVULSANT DRUGS IN RATS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE RIJKSUNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS DR. L. LEERTOUWER, HOOGLERAAR IN DE FACULTEIT DER GODGELEERDHEID, VOLGENS BESLUIT VAN HET COLLEGE DER DEKANEN TE VERDEDIGEN OP DONDERDAG 12 DECEMBER 1991 TE KLOKKE 14.15 UUR

DOOR

ANNA MARIA STIJNEN

GEBOREN TE PEIJ-ECHT IN 1964

1991
DRUKKERIJ DEN HOED,
WIJK BIJ DUURSTEDE

Promotiecommissie:

Promotor:	Prof. dr. D.D. Breimer
Co-promotores:	Dr. M. Danhof
	Dr. C.F.A. van Bezooijen
Referent:	Prof. dr. E.R. de Kloet
Overige leden:	Prof. dr. G.J. Mulder
	Prof. dr. W. Soudijn
	Prof. dr. D.L. Knook

na zoveel wijsheid
danst eindelijk de sleutel in het slot
en kraakt de deur zich open
hoeveel boeken heeft dat niet gekost
en de tijd

doch waar de wonderen rinkelen
blijven vragen als goddelijke poorten
gesloten
schuilt daar de vrede thans
dat ik mijn simpel vragen heb gestaakt

(Laurens Boom)

In herinnering aan Suzanne
en voor mijn familie en vrienden

The investigations described in this thesis were performed at the Division of Pharmacology, Center for Bio-Pharmaceutical Sciences, Leiden University and the former TNO Institute for Experimental Gerontology, Rijswijk, The Netherlands, now part of the TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands.

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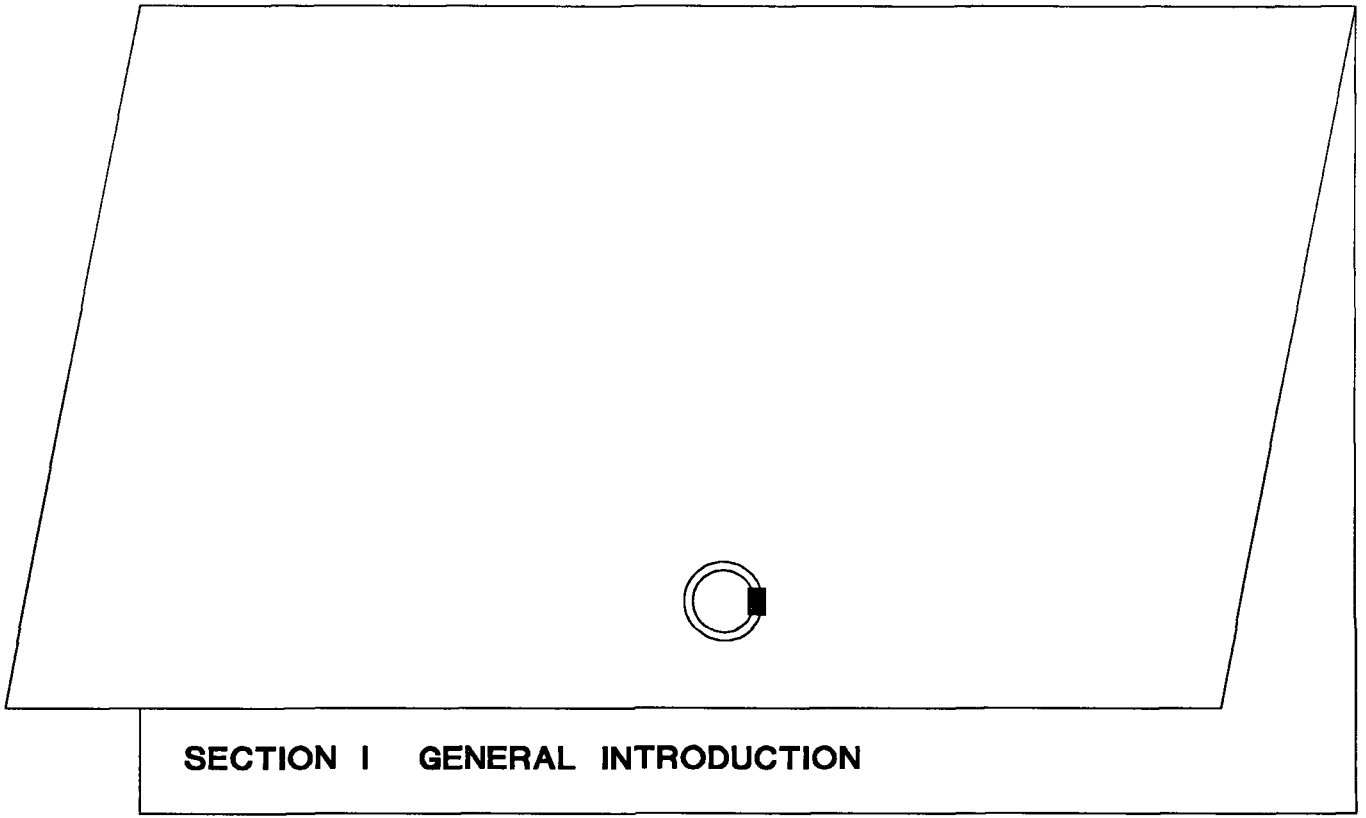
THE INFLUENCE OF AGEING ON THE PHARMACODYNAMICS OF SEDATIVE AND ANTICONVULSANT DRUGS IN RATS

CONTENTS



SECTION I GENERAL INTRODUCTION

Chapter 1

THE INFLUENCE OF AGEING ON THE PHARMACODYNAMICS OF DRUGS ACTING ON THE CENTRAL NERVOUS SYSTEM

Summary

The number of elderly people in our society increases steadily. The percentage of this age group, that requires drug treatment is relatively high compared to young people. In addition, the elderly are confronted with a relatively high incidence of adverse drug reactions. The possible mechanisms behind this, in terms of pharmacokinetics and pharmacodynamics, have only been partially investigated. Age-related changes in pharmacokinetics are now reasonably well documented, potential changes in pharmacodynamics are however still relatively unexplored.

In this chapter, special attention is paid to age-related changes in pharmacodynamics of barbiturates and benzodiazepines as model compounds for drugs acting on the central nervous system. For these compounds, a decrease in dose requirement with increasing age was observed in studies in man. Several of those studies also showed indications for an age-related increase in brain sensitivity, whereas other studies did not. However, the interpretation of these indications is often difficult, due to changes in baseline effect during ageing or to possible age-related interfering pharmacokinetic changes, that are not taken into consideration, e.g. changes in distribution of the drug between plasma and site of action or in the formation of (inter)active metabolites.

In in vivo studies in animals an increase in brain sensitivity to barbiturates and benzodiazepines during ageing has been reported. Nevertheless also controversial results have been obtained. Also in the studies in animals, baseline effect values showed age-related changes and interfering pharmacokinetic factors were not always taken into consideration. Moreover, the health status of the animals has not adequately been assessed in such

studies. This is essential because observed changes during ageing may in actual fact be due to age-associated pathology.
in vitro pharmacodynamic studies have not provided sufficient insight into the possible mechanisms behind an increased brain sensitivity during ageing, because of their contradictory nature.
It is concluded that more extensive and rigorously designed pharmacodynamic studies, including investigations into age-related changes in baseline effect, age-related pathology and pharmacokinetic changes, are needed to enlarge our insight into the effect of ageing on pharmacodynamics of drugs acting on the central nervous system. Studies in animal models of ageing may be particularly valuable in this respect.

1. Introduction

The elderly constitute a still growing percentage of our population; the absolute number of people of 65 years and over is also increasing (Stevenson, 1984; Van Bezooijen, 1985; Klotz, 1986; Bunker and Clayton, 1989; Holden, 1990). Since the incidence of pathology is positively correlated with age (Hauser and Kurland, 1975; Pucino et al., 1985; Brody and Schneider, 1986; Miller, 1987), the elderly consume a disproportionately high share of drugs (Salzman, 1982; Stevenson, 1984; Everitt and Avorn, 1986; Klotz and Brückel, 1982; Klotz, 1986; van Bezooijen, 1989; Tregaskis and Stevenson, 1990). Next to the high consumption of drugs, the elderly appear to be more sensitive to develop adverse drug reactions than younger subjects after administration of the same dose levels (Bender, 1974; Castleden et al., 1977; Greenblatt et al., 1977; Greenblatt and Allen, 1978; Gordon and Preiksaitis, 1988; Montamat et al., 1989). Not only the relationship between the dose of a drug and the unwanted adverse reactions changes during ageing, this holds also true for the relationship between the dose and the therapeutic effect (Reidenberg et al., 1978; Ouslander, 1981; Stevenson, 1984; Bell et al., 1987; Miller, 1987). Dose adjustment in the elderly appears often to be indicated. As can be seen in figure 1, the relationship between dose of a drug and the pharmacological or adverse effects is determined by both pharmacokinetic processes (distribution, metabolism and excretion) and pharmacodynamic processes (drug receptor interaction, effectuation processes and homeostatic mechanisms). The cause for differences in dose requirement between young and elderly subjects can therefore be found in

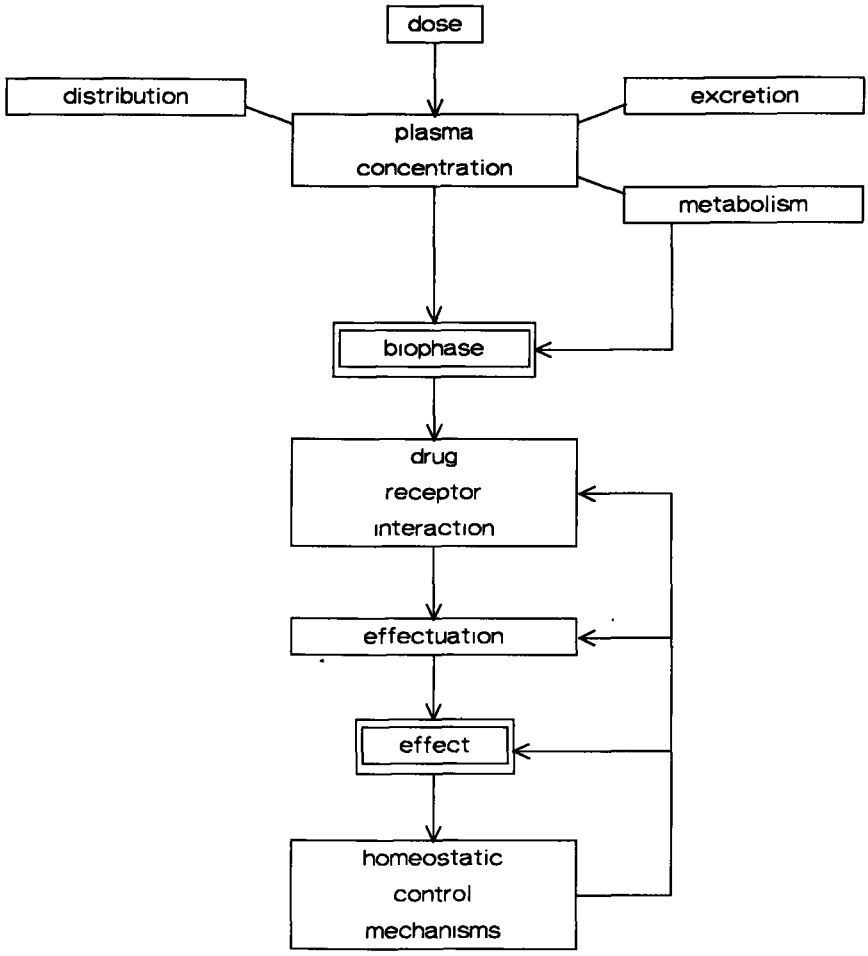


Figure 1. Pharmacokinetic and pharmacodynamic processes which determine the relationship between the administered dose and the resulting effect intensity of a drug.
After administration the drug is distributed in the body, metabolized and/or excreted. These pharmacokinetic processes determine the concentration of the drug in the biophase. There the drug interacts with the receptor and via effectuation processes the pharmacological or adverse effect is accomplished. The effect intensity is influenced by homeostatic mechanisms. If a drug is metabolized into an active compound which also reaches the biophase, this metabolite determines part of the total effect intensity.

changes in both pharmacokinetics and pharmacodynamics during ageing (Bender, 1974; Scott, 1982; Ho and Triggs, 1984; Lamy, 1987; Miller, 1987; Roberts and Turner, 1988, 1988a; Montamat et al., 1989; Tsujimoto et al., 1989, 1989a). These changes can be enhanced by several kinds of concomitant diseases (Kato, 1977; Greenblatt et al., 1977; Greenblatt and

Table 1. *Age-related changes in response to drugs acting on the central nervous system in man.*

drug	dose requirement	pharmacokinetic findings	pharmacodynamic findings	references
anaesthetics/ sedatives				90
propofol	↓			32
thiopental	↓	V_{ss} ↑ or =, V_c ↓ or =, Cl =	Unchanged brain sensitivity (on basis of EEG analysis and of threshold concentrations for loss of eyelash reflex)	5, 20, 21, 22, 61, 127
diazepam	↓	V_{ss} ↑, Cl ↓ or =	Decreased threshold concentration for defined degree of sedation	24, 40, 110, 135
midazolam	↓ or = (females)	V, V_{ss} ↑ or =, Cl ↓ (males)		6, 47, 123
etomidate	↓	V_{ss} =, V_c ↓, Cl =	Unchanged brain sensitivity (on basis of EEG analysis)	2
antidepressants				98
nortriptyline	↑	Cl ↓		34, 39
analgetics				65, 98
alfentanil	↓	No changes	Increased brain sensitivity (on basis of EEG analysis)	122
fentanyl	↓	No changes	Increased brain sensitivity (on basis of EEG analysis)	122
bupivacaine	↓	Cl ↓		144, 145
morphine	↓	V ↓ or =, Cl ↓ or =		34, 64, 65
pentazocine	↓			7
CNS stimulants	↑			116
amphetamine				9
neuromuscular blocking agents				
vecuronium	↓ or =	V_{ss} ↓, Cl ↓	unchanged	97, 115
pancuronium	↓	Cl ↓ or =	unchanged	35, 115
tubocurarine	↓	V_c ↓, V ↓, Cl ↓	unchanged	90
metocurine	↓	V_c ↓, V ↓, Cl ↓	unchanged	90
atracurium	=			90
anticholinesterase inhibitors				
neostigmine	↓			149
pyridostigmine	↓			149
anticholinergic agents				
atropine	↓	Cl ↓		124, 146

V: volume of distribution
V_{ss}: steady state volume of distribution
V_c: volume of the central compartment
Cl: total body clearance

↑: increase with increasing age
↓: decrease with increasing age
=: no change during ageing

Allen, 1978; Murray et al., 1981; Stevenson, 1984). Since many elderly need more than one drug at the same time, drug interactions also attribute to the differences in intensity of pharmacological and adverse drug effects in this group compared to younger subjects (Bender, 1979; Roberts and Turner, 1988a). In this respect, the fact that the etiology of an illness can change during ageing may be an important issue. Changes in sleep pattern (Roehrs et al., 1985) and pattern of depressions (Friedel, 1981) for example can give rise to a change in the pharmacodynamics of hypnotics and antidepressants.

2. Age-related changes in dose requirement of drugs acting on the central nervous system

Among all drugs used by the elderly, cardiovascular agents and drugs acting on the central nervous system (CNS) are the most frequently prescribed (Haaijer-Ruskamp and Dingemans, 1989). In the following we will focus on CNS active drugs. Within this group, the use of benzodiazepines predominates (Haaijer-Ruskamp and Dingemans, 1989). Table 1 shows several examples of decreased or increased dose requirement of CNS active drugs in the elderly, supplemented with data on changes in pharmacokinetics and pharmacodynamics. It is clear that the dosage regimens in the elderly patients need often to be adjusted.

Concerning the processes underlying the change in dose requirement during ageing, research has improved considerably our knowledge of age-related changes in pharmacokinetics and their physiological background (Ouslander, 1981; Greenblatt et al., 1982; Klotz and Brückel, 1982; Lamy, 1982; Ho and Triggs, 1984; Stevenson and Hosie, 1985; Van Bezooijen, 1986; Ritschel, 1987; Roberts and Turner, 1988; Montamat et al., 1989; Tsujimoto et al., 1989; Durnas et al., 1990; Tresgaskis and Stevenson, 1990; Birnbaum, 1991; Groen, 1991). In brief, due to the decrease of the total amount of body fluid and the increase in fatty tissue, the volume of distribution increases with age for lipophilic and decreases for hydrophillic drugs. Since kidney function deteriorates during ageing, the renal clearance of several drugs and metabolites shows an age-related decrease. Because of a decrease in activity of several metabolizing enzymes, the metabolism of several drugs, especially those which are metabolized via phase I metabolic reactions, and of some drugs, for which phase II reactions are involved, declines during ageing. It should be realized, however, that the age-related differences in

Table 2. *The influence of ageing on pharmacodynamics of barbiturates and benzodiazepines in man.*

drug	effect measured	findings	references
phenobarbital	drowsiness (subjective)	incidence =	14
thiopental	sedation	D ↓	32, 93
	sedation	D ↓; threshold plasma concentration =	5, 20, 21, 22
	EEG Effect	D ↓; E_{max} =; EC_{50} =; E_0 =	61, 127
chlordiazepoxide	drowsiness (subjective)	incidence ↑	14
diazepam	sedation	D ↓; threshold plasma concentration ↓	24, 40, 110
	sedation (subjective)	E =; unbound plasma concentration ↓	135
	sedation (subjective)	E ↑; plasma concentration ↑	104
	drowsiness (subjective)	incidence ↑	14
	psychomotor impairment	E ↑; baseline psychomotor performance ↓	103, 104
	psychomotor impairment	E ↑; baseline psychomotor performance =, plasma concentration = or ↓	25
	psychomotor impairment	E =; baseline psychomotor performance ↓	58
	psychomotor impairment	body sway: E ↑ and baseline ↑; digit symbol substitution: E = and baseline performance ↓, E/baseline ↑; unbound plasma concentration ↓	135
	memory impairment	E ↑; plasma concentration ↑; baseline performance ↓	104
	memory impairment	recall: E = and baseline performance ↓; cognitive processes: E ↑ and baseline performance ↓	105
	memory impairment	E =; baseline performance ↓	58
flunitrazepam	sedation	E ↑; threshold plasma concentration =	66, 82
	amnesia	E ↑	82
flurazepam	drowsiness, confusion, ataxia (subjective)	incidence ↑ only at high dosages (>15 - 30 mg)	43, 45
loprazolam	sedation (subjective)	E ↑, plasma concentration =	134
	psychomotor impairment	E ↑, baseline psychomotor performance ↓, plasma concentration =	134
midazolam	sedation	D ↓	6
nitrazepam	drowsiness, hangover (subjective)	incidence ↑	18, 44
	nightmares, insomnia, agitation (subjective)	incidence =	44
	psychomotor impairment	Accuracy: E ↑ and baseline performance ↓, speed: E = and baseline performance ↓	18, 19 136

continued

drug	effect measured	findings	references
temazepam	sedation (subjective)	E ↑; plasma concentration =	27, 132
	psychomotor impairment	Body sway and choice reaction time: E ↑ and baseline performance ↓ and ↑, respectively; critical flicker fusion time: E ↑ and baseline performance =; plasma concentration =	27, 128, 132
triazolam	sedation (subjective)	Concentration vs. effect relationship: slope regression line =	48
	psychomotor impairment	Concentration vs. effect relationship: slope regression line = (effect expressed as percentage of baseline level); baseline performance ↓	48
	memory impairment	E =; (effect expressed as percentage of baseline level); baseline performance ↓	48

E: drug effect intensity
D: dose requirement
E_{max}: maximum effect
EC_{50}: concentration needed to reach half the maximum effect
E_0: baseline effect

↑: increase with increasing age
↓: decrease with increasing age
=: no change during ageing

pharmacokinetics may not always be considerable; smoking habits and diseases seem to have a much greater impact (Kato, 1977; Crooks and Stevenson, 1981; Stevenson, 1984; Gordon and Preiksaitis, 1988; Montamat et al., 1989). All these factors together bring about an increase in interindividual variability in pharmacokinetics increases during ageing. Compared to the relative abundance of pharmacokinetic information in the elderly, data on age-related changes in pharmacodynamics are scarce (Roberts and Turner, 1988; Montamat et al., 1989).

3. Pharmacodynamics of barbiturates and benzodiazepines during ageing in man

A decreased dose requirement during ageing has been reported for several barbiturates and benzodiazepines (table 2). Because of this fact, combined with the frequent use of benzodiazepines in the elderly and the use of barbiturates and benzodiazepines as model compounds in the studies described in this thesis, we will concentrate on barbiturates and benzodiazepines in the following.

For some of the barbiturates and benzodiazepines the available information on age-related changes in dose requirement only consists of an increased frequency of reported adverse effects; for others sedation, psychomotor impairment or memory impairment have been assessed more objectively. For

thiopental, diazepam, flunitrazepam, loprazolam, nitrazepam and temazepam the pharmacodynamics were studied during ageing (table 2). The pharmacodynamics of thiopental were found not to change during ageing on the basis of both plasma threshold concentrations for sedation (Christensen et al. 1982, 1983) and maximal EEG effect values and steady state plasma concentrations at half maximum EEG effect (Homer and Stanski, 1985; Stanski and Maitre, 1990). However, for the four benzodiazepines, mentioned above, an increase in brain sensitivity with increasing age was observed. The diazepam threshold plasma concentration for sedation was negatively correlated with age (Reidenberg et al., 1978; Cook et al., 1984) and after administration of a fixed dose of diazepam the effect on psychomotor impairment was higher in the elderly with comparable or lower plasma concentrations (Swift et al., 1985a; Cook, 1986). For flunitrazepam a higher degree of sedation was observed in elderly subjects compared to younger ones, whereas the plasma concentrations were comparable for both age groups (Kanto et al., 1981). Finally, similar plasma concentrations in young and elderly subjects of loprazolam, nitrazepam and temazepam were found to result in a higher degree of psychomotor impairment in the elderly (Castleden et al., 1977; Castleden and George, 1979; Swift et al., 1981, 1985; Stevenson et al., 1982; Crooks, 1983).

Complexities of studies on pharmacodynamics in the elderly

The interpretation of the results of the studies discussed so far, however, is not unambiguous. Especially for the measurement of the effect of drugs on cognitive and psychomotor performance and on learning and memory, it should be realized that the baseline performance may change during ageing. The baseline body sway and the baseline critical reaction time show an age-related increase (Swift et al., 1981; Stevenson et al., 1982; Crooks, 1983; Swift et al., 1984, 1985, 1985a; Pomara et al., 1985; Hinrichs and Ghoneim, 1987). The baseline performance for the digit symbol substitution test and both the speed and accuracy of the letter e deletion test is declining during ageing (Castleden et al., 1977; Castleden and George, 1979; Swift et al., 1985a), whereas the baseline performance of the critical flicker fusion test does not differ between young and elderly subjects (Swift et al., 1981; Crooks, 1983). The memory functioning is decreasing during ageing (Hinrichs and Ghoeneim, 1987; Pomara et al., 1989). When baseline values are different for young and old subjects, the drug effect can be expressed as an

absolute effect or as a percentage of the baseline value. This can lead to conflicting results, as is reported for the effect of diazepam on the digit symbol substitution test, which is only statistically significant when values are expressed as a percentage of the baseline value (Swift et al., 1985a). In some instances the degree of the baseline cognitive and psychomotor motor performance in the elderly is even lower than the performance in the young after administration of a benzodiazepine (Castleden, 1977; Hinrichs and Ghoneim, 1987). The latter authors measured the effect of diazepam on learning and memory and on cognitive and psychomotor tasks and reported that the decrements in performance were about the same in young, middle-aged and old subjects, but that the baseline performance decreased with increasing age. This lower baseline performance may make equal decrements more noticeable and more serious in the elderly. It is therefore questionable, whether the reported increase in sensitivity to benzodiazepines in the elderly is due to a higher sensitivity to the drug effect or to a decrease in baseline performance.

The interpretation of the pharmacodynamic studies in the elderly can be complicated by the following pharmacokinetic factors: age-related differences in plasma protein binding, changes in the ratio between the concentrations in plasma and at the site of action, and the role of (inter)active metabolites and/or enantiomers (Dingemanse et al., 1988). In most studies, the total plasma concentration of the drug is measured. However, since only unbound drug molecules in plasma can diffuse to the brain (the site of action of the barbiturates and benzodiazepines), the plasma protein binding can be an important parameter in evaluating the relationship between total plasma concentration and drug effect in different age groups. Age-related decreases in plasma protein binding have been reported for thiopental, diazepam and nitrazepam (Klotz, 1986; Wallace and Verbeeck, 1987). Changes in protein binding can be caused by the age-related decline of the albumin concentration or increase of the α_1-acid glycoprotein concentration (Greenblatt et al., 1982; Ritschel, 1987; Wallace and Verbeeck, 1987). Concerning the observed decrease of the albumin concentration in some studies, it has been reported that this is due to age-related pathology or poor nutrition. In general, the albumin concentration in healthy elderly is not different from that in younger people (Everitt and Avorn, 1986; Montamat et al., 1989).

In rats and mice an age-related increase in the concentration ratio brain/plasma has been reported for pentobarbital, diazepam, nitrazepam and

oxazepam (Hewick and Shaw, 1978; Pardon and Jones, 1978; Rahman et al., 1986; Kitani et al., 1989). The basis for this increase might be an increase in non-specific binding to brain tissue, but also to age-related physiological changes in the blood-brain barrier, although literature data on these changes and their pharmacological implications are contradictory (Rapoport et al., 1979; Banks and Kastin, 1988; Harik, 1988; Mooradian, 1988; Pardridge, 1988).

Because several barbiturates and in particular benzodiazepines are metabolized to pharmacologically active compounds, these metabolites can be responsible for part of the measured activity (figure 1). In several investigations on pharmacodynamics and ageing metabolites are not taken into account, although exposure to (inter)active metabolites may change during ageing (Durnas et al., 1990; Birnbaum, 1991).

In many studies on age-related changes in pharmacodynamics the drugs under investigation consist of a racemic mixture, which hampers the interpretation of the outcome of these studies, since the observed pharmacological response is the result of two or more compounds with qualitatively or quantitatively different pharmacological properties acting on the same physiological system (Drayer, 1986; Schüttler et al., 1987).

4. Animal studies on age-related changes in *in vivo* pharmacodynamics

In order to further investigate the mechanism behind the decrease in dose requirement for barbiturates and benzodiazepines in the elderly in terms of pharmacokinetics and pharmacodynamics, several *in vivo* studies in rats and mice have been performed (table 3). In experimental animals it is possible to measure brain and cerebrospinal fluid concentrations. Moreover, *in vitro* studies can be performed to gain more insight into the mechanism of age-related pharmacodynamic changes. The reason for the age-related decrease in anaesthetic dose requirement of barbital, hexobarbital and pentobarbital (which is not observed in all rat strains) was found to be a decrease in metabolic activity, whereas the brain sensitivity was presumably unchanged during ageing (Hewick, 1979). For phenobarbital the decrease in anaesthetic dose requirement was suggested to be (at least in part) due to an increased brain sensitivity (Wanwimolruk and Levy, 1987). On the basis of hexobarbital-induced EEG changes, both an age-related increased brain sensitivity or no

Table 3. *The effect of ageing on pharmacodynamics of barbiturates and benzodiazepines in rats and mice.*

drug	effect measured	findings	references
barbital	anaesthesia	Sleeping time ↑; brain concentration at end of sleeping time = (in Wistar rat)	57
	anaesthesia	Sleeping time ↑ (in C57BL/6 mouse and New Zealand Black mouse)	38
hexobarbital	anaesthesia	Sleeping time ↑; metabolic activity ↓; rate of elimination from plasma and brain ↓; Wistar rat	57
	anaesthesia	Sleeping time = (in Sprague-Dawley rat)	131
	anaesthesia	Sleeping time ↑ (in C57BL/6 mouse and New Zealand Black mouse)	38
	EEG effect	Threshold dose ↓, threshold brain concentration ↓ (in Sprague-Dawley rat)	13
	EEG effect	Threshold dose ↓, threshold brain concentration = (in Cox/Sprague-Dawley rat)	118
pentobarbital	anaesthesia	Sleeping time ↑; metabolic activity ↓; brain concentration at end of sleeping time = (in Wistar rat)	57
	anaesthesia	Sleeping time = (in Long Evans rat)	50
phenobarbital	anaesthesia	Threshold dose and threshold cerebrospinal fluid concentration ↓	148
	anticonvulsant effect	ED_{50} ↓; minimal effective brain concentration ↓	68, 70, 71, 102
	ataxia	Minimal effective brain concentration ↓	71
clonazepam	ataxia	E at any given brain concentration or degree of receptor occupancy ↑	4
diazepam	anaesthesia	Sleeping time ↑; brain concentration of diazepam and desmethyldiazepam at end of sleeping time =	50
	learning and memory	E ↑	76
	ataxia	E ↑	38
	anticonflict test	E ↑; threshold dose ↓	79
oxazepam	anticonvulsant effect	Minimal effective brain concentration ↓	72
	ataxia	Minimal effective brain concentration ↓	72
temazepam	passive avoidance	E ↑	81

E: drug effect intensity
ED_{50}: effective dose in 50% of the animals

↑: increase with increasing age
↓: decrease with increasing age
=: no change during ageing

change were reported (Saunders et al., 1974; Bolander and Wahlström, 1984). The rats used in those studies were relatively young though (aged

between 1.5 and 10 months). The age-related decrease in minimal effective anticonvulsant phenobarbital and oxazepam brain concentration (Kitani et al., 1985, 1986, 1988, 1989) could be reflecting either an increased brain sensitivity or a decrease in baseline sensitivity to convulsant agents (Kitani et al., 1985a; Nokubo et al., 1986) or a combination of both. For diazepam an increase in effect intensity during ageing was measured for four different effects (anaesthesia, learning and memory, ataxia and anticonflict) (table 3). It should be realized, however, that the baseline learning and memory performance and the baseline for the anticonflict test used show age-related variations (Amrick and Bennett, 1987; Komiskey et al., 1987; McMahon et al., 1987). Only for the anaesthetic effect, concentrations were measured, resulting in no age-related differences in brain and serum diazepam and desmethyldiazepam concentrations, providing no indications for a change in brain sensitivity. The greater ataxia after clonazepam administration in middle-aged mice is presumably in part due to an increased brain sensitivity, which, however, could not be explained by changes in benzodiazepine receptor binding, chloride channel binding or gamma-aminobutyric acid-dependent muscimol-stimulated chloride uptake (Barnhill et al., 1990).
A major drawback of most studies in animals performed until now is the fact, that the health status of the animals has not been assessed. Observed changes during ageing may therefore be due to age-associated pathology.

5. *In vitro* pharmacodynamic studies

Changes in pharmacodynamics can be a result of changes in drug-receptor interaction, post-receptor events (effectuation processes) or in homeostatic mechanisms (figure 1). The overall pharmacological effect is accomplished by the total of these processes and can be studied *in vivo*. The separate processes can be investigated *in vitro*. In this paragraph, first the *in vitro* studies on benzodiazepine pharmacodynamics will be reviewed, followed by those on barbiturates.

5.1. Benzodiazepines

Benzodiazepines exert their pharmacological effect via the γ-aminobutyric acid-benzodiazepine receptor complex (Schwartz, 1988; Haefely, 1989). In figure 2 it is shown that this receptor complex consists of several functionally coupled subunits. Binding of γ-aminobutyric acid (GABA) to the GABA

Table 4a. *In vitro pharmacodynamics of benzodiazepines during ageing: benzodiazepine receptor binding.*

brain area	total binding at one ligand concentration	receptor density	receptor affinity	references
whole brain		=	=	4, 55
cerebellum	↓			77
		↓ or = or ↑	=	17, 28, 62, 101
cortex	↓			77
		↓ or =	↓ or =	17, 89, 95, 101, 109, 138
hippocampus	↓			77
		↓ or ↑	=	88; 17; 28
striatum		↑	=	17

↑: increase with increasing age
↓: decrease with increasing age
=: no change during ageing

receptor gives rise to opening of the chloride channel and benzodiazepines potentiate the effect of GABA, without changing the maximum GABA effect, by binding to the benzodiazepine receptor. The effects of age on the benzodiazepine receptor binding, the GABA receptor binding, the chloride channel binding and the functional interaction between these subunits of the GABA-benzodiazepine receptor complex have been investigated and are discussed in successive order in this section.

5.1.1. Benzodiazepine receptor binding (table 4a)

In vitro benzodiazepine receptor affinity appears not to change with age even if differences in regional distribution are taken into consideration (only in one study a small decrease in affinity was reported (Meyers and Komiskey, 1985)). This is in contrast to the reported age-related decrease in *in vivo* receptor binding affinity (Barnhill et al., 1990). *In vitro* receptor density does not change in whole brain homogenates, increases during ageing in striatum (only one study), and the results on cerebellum, cortex and hippocampus are varying (for cortex only one study showed a decrease, the others no changes) (for references: see table 4a). Komiskey and MacFarlan (1983) observed a decrease in total binding at one ligand concentration in cerebellum, cortex and hippocampus. Reeves and Schweizer (1983) reported a higher increase in benzodiazepine receptor density in old animals after acute diazepam pretreatment compared to that in adult young ones and Komiskey (1987) measured a decreased *ex vivo* ^{3}H flunitrazepam binding after acute diazepam

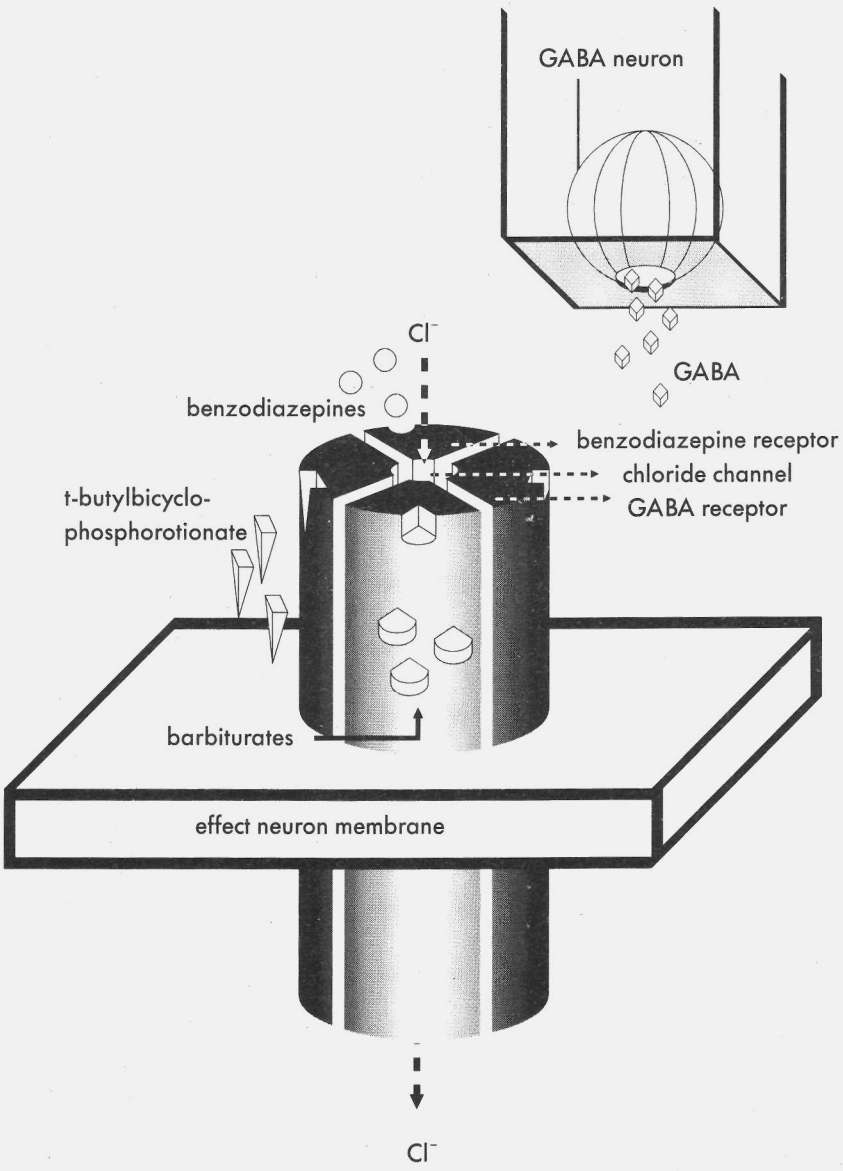


Figure 2. Schematic representation of the GABA-benzodiazepine receptor complex.
The receptor complex consists of several functionally coupled subunits. Binding of γ-aminobutyric acid (GABA) to the GABA receptor leads to opening of the chloride channel. Benzodiazepines potentiate the effect of GABA (without changing the maximum effect) by binding to the benzodiazepine receptor. Barbiturates are believed to bind to a separate subunit. T-butylbicyclophosphorotionate binds to a protein, which is functionally coupled to the chloride channel, and is used as a ligand to study chloride channel binding.

Table 4b. *In vitro pharmacodynamics of benzodiazepines during ageing: GABA receptor binding.*

brain area	total binding at one ligand concentration	receptor density	receptor affinity	references
cerebellum low affinity binding sites		=	↑	62
high affinity binding sites		↑	=	62
cortex low affinity binding sites		↓	=	95
high affinity binding sites		=	=	95
cerebellum	=			42, 96
brain tissue above cerebellum		=	=	80
cortex	=			42, 96
		=	=	36
hippocampus	=			28
		=	=	85
brain stem, striatum, nucleus accumbens	=			42, 96
hypothalamus, substantia nigra	↓			42

↑: increase with increasing age
↓: decrease with increasing age
=: *no change during ageing*

pretreatment only in senescent rats.
Taking these studies together, the results are not conclusive for an age-related change in benzodiazepine receptor binding characteristics during ageing.

5.1.2. GABA receptor binding (table 4b)
Depending on the brain area and the kind of binding site (low or high affinity) the GABA receptor affinity was reported to remain constant or increase during ageing; for the receptor density an increase, a decrease or no changes were measured (for references: see table 4b).

5.1.3. Chloride channel binding (table 4c)
Depending on the brain area the receptor density of the binding site for t-butylbicyclophosphorotionate (which is located on a protein which is functionally coupled to the chloride channel, see figure 2) was found to decrease or to remain constant during ageing and the receptor affinity remained intact during the ageing process (for references: see table 4c).

Table 4c. *In vitro pharmacodynamics of benzodiazepines during ageing: chloride channel binding.*

brain area	receptor density	receptor affinity	references
whole brain	=	=	4
cortex	↓	=	23, 36

↓: decrease with increasing age
=: no change during ageing

5.1.4. Functional interaction between the benzodiazepine receptor, GABA receptor and chloride channel (table 4d)

The effect of GABA on benzodiazepine receptor binding was reported to decrease or remain constant during ageing. An increase or no change with age was found for the effect of benzodiazepines on GABA receptor binding, whereas the effect of GABA and of benzodiazepines on the function of the chloride channel were measured to remain constant or decrease during ageing (for references: see table 4d).

5.1.5. Endogenous GABA concentration

The endogenous cerebral cortex GABA concentration was reported not to show age-related changes (Meyers and Komiskey, 1985). No data are available on the GABA concentrations during ageing in other brain areas.

In general, it is concluded that the results obtained in the *in vitro* studies on benzodiazepine pharmacodynamics are not unambiguous. This may be due to differences in the rat or mouse strain used and to differences in experimental procedures (e.g. homogenization and purification procedures, incubation temperature). Overviewing the data on *in vitro* pharmacodynamic studies concerning the GABA-benzodiazepine receptor complex, no consistent evidence is available for important age-related changes in the receptor complex.

5.2. Barbiturates

Meyers and Komiskey (1985) investigated the modulation of [^{3}H]flunitrazepam receptor binding by pentobarbital and did not find age-related changes in the effect of this barbiturate.

Jones and Beaney (1980) measured an age-related decrease in the depressant effect of pentobarbital on the uptake of Ca^{2+} and release of

Table 4d. *In vitro pharmacodynamics of benzodiazepines during ageing: functional interaction between benzodiazepine receptor, GABA receptor and chloride channel.*

finding		references
effect GABA on increase in benzodiazepine receptor binding		
whole brain:	=	55
cerebellum:	↓ or =	17, 75
cortex:	=	75
hippocampus:	=	75
effect benzodiazepine on increase in GABA receptor binding		
cerebellum:	↑	17
brain tissue above cerebellum:	=	80
effect GABA on chloride channel responsiveness		
whole brain:	=	4
cortex:	↓	23, 36
effect benzodiazepine on chloride channel responsiveness		
whole brain:	=	4
cortex:	↓	23

↑: increase with increasing age
↓: decrease with increasing age
=: no change during ageing

neurotransmitters of neuronal terminals isolated from brain homogenates. This process presumably reflects the mechanism via which the barbiturates induce anaesthesia. Desbarats-Schonbaum and Birmingham (1959) reported an age-related decrease in the depressing effect of pentobarbital on tissue respiration in brain homogenates. This effect of pentobarbital appears to be correlated with its anaesthetic effect. The decreasing effect of the barbiturates during ageing, found in these two studies, is in contrast with the age-related increase in the *in vivo* anaesthetic effect of barbiturates (Christensen et al., 1982; Stanski and Maitre, 1990). Thus the *in vitro* studies do not elucidate the mechanism behind the increasing anaesthetic effect *in vivo* of barbiturates during ageing.

One important problem in performing *in vitro* studies to elucidate the mechanism behind age-related pharmacodynamic changes *in vivo*, is the fact, that in *in vitro* studies not all control mechanisms are operating. As a result of age-related impairment in homeostatic mechanisms, a higher susceptibility for drug effects is expected in the elderly, giving rise to postural hypotension, hypothermia, dizziness, confusion, agitation, weakness, change in glucose

levels and decline in neurological control of bladder and bowel function (Shock, 1961, 1983; Bender, 1969, 1974; Crooks, 1983; Stevenson, 1984; Roberts and Turner, 1988a). Therefore, the functional levels of such homeostatic mechanism are likely to be important in determining drug effects in the elderly.

6. Conclusions

In vivo pharmacodynamic studies in man and animals suggest an age-related increase in the brain's sensitivity to barbiturates and benzodiazepines. The question whether the sensitivity changes quantitatively to a significant degree during ageing still remains, however. *In vitro* studies have not elucidated the mechanism behind the possible change in sensitivity. Therefore, more extensive and rigorously designed pharmacodynamic studies are needed to enlarge our insight into the effect of ageing on the sensitivity of the brain to barbiturates and benzodiazepines. Investigations into age-related changes in baseline effect and pharmacokinetic changes have to be included in these studies. Studies in animal models of ageing may be particularly valuable in this respect.

7. References

1. Amrick, C.L. and Bennett, D.A.: A comparison of diazepam stimuli in aged and adult rats. Psychopharmacol. 93: 292-295, 1987.

2. Arden, J.R., Holley, F.O. and Stanski, D.R.: Increased sensitivity to etomidate in the elderly: initial distribution versus altered brain response. Anesthesiol. 65: 19-27, 1986.

3. Banks, W.A. and Kastin, A.J.: Peptides and the senescent blood-brain barrier. Neurobiol. Aging 9: 48-49, 1988.

4. Barnhill, J.G., Greenblatt, D.J., Miller, L.G., Gaver, A., Hartmatz, J.S. and Shader, R.I.: Kinetic and dynamic components of increased benzodiazepine sensitivity in aging animals. J. Pharmacol. Exp. Ther. 253: 1153-1161, 1990.

5. Becker, K.E.: Plasma levels of thiopental necessary for anesthesia. Anesthesiol. 49: 192-196, 1978.

6. Bell, G.D., Spickett, G.P., Reeve, P.A., Morden, A. and Logan, R.F.A.: Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. Br. J. clin. Pharmacol. 23: 241-244, 1987.

7. Bellville, J.W., Forrest, W.H., Miller, E. and Brown, B.W.: Influence of age on pain relief from analgesics: A study of postoperative patients. J. Am. Med. Assoc. 217: 1835-1841, 1971.

8. Bender, A.D.: Pharmacologic aspects of aging. A survey of the effect of increasing age on drug activity in adults. J. Am. Geriatr. Soc. 12: 114-134, 1964.

9. Bender, A.D.: Pharmacodynamic principles of drug therapy in the aged. J. Am. Geriatr. Soc. 22: 296-303, 1974.

10. Bender, A.D.: Geriatric pharmacology. Age and its influence on drug action in adults. Drug Inf. Bull. 3: 153-158, 1969.

11. Bender, A.D.: Drug sensitivity in the elderly. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson, eds., The Macmillan Press Ltd., 1979, pp 147-153.

12. Birnbaum, L.S.: Pharmacokinetic basis of age-related changes in sensitivity to toxicants. Ann. Rev. Pharmacol. 31: 101-128, 1991.

13. Bolander, H.G. and Wahlström, G.: Age-related changes in CNS-sensitivity to hexobarbital and thiopental in the rat. Arch. Int. Pharmacodyn. Thér. 267: 213-223, 1984.

14. Boston Collaborative Drug Surveillance Program. Clinical depression of the central nervous system due to diazepam and chlordiazepoxide in relation to cigarette smoking and age. New Engl. J. Med. 288: 277-280, 1973.

15. Brody, J.A. and Schneider, E.L.: Diseases and disorders of aging: an hypothesis. J. Chron. Dis. 39: 871-876, 1986.

16. Bunker, V.W. and Clayton, B.E.: Research review: Studies in the nutrition of elderly people with particular reference to essential trace elements. Age and Ageing 18: 422-429, 1989.

17. Calderini, G., Bonetti, A.C., Aldinio, A., Savioni, G., Di Perri, B., Biggio, G. and Toffano, G.: Functional interaction between benzodiazepine and GABA recognition sites in aged rats. Neurobiol. Aging 2: 309-313, 1981.

18. Castleden, C.M., George, C.F., Marcer, D. and Hallett, C.: Increased sensitivity to nitrazepam in old age. Br. Med. J. 1: 10-12, 1977.

19. Castleden, C.M. and George, C.F.: Increased sensitivity to benzodiazepines in the elderly. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson, eds., The Macmillan Press Ltd., 1979, pp 169-177.

20. Christensen, J.H. and Andreasen, F.: Individual variation in response to thiopental. Acta

Anaesthesiol. Scand. 22: 303-313, 1978.

21. Christensen, J.H., Andreasen, F. and Jansen, J.A.: Pharmacokinetics and pharmacodynamics of thiopentone. A comparison between young and elderly patients. Anaesthesia 37: 398-404, 1982.

22. Christensen, J.H., Andreasen, F. and Jansen, J.A.: Thiopentone sensitivity in young and elderly women. Br. J. Anaesth. 55: 33-40, 1983.

23. Concas, A., Pepitoni, S.J., Atsoggiu, T., Toffano, G. and Biggio, G.: Aging reduces the GABA dependent ^{36}Cl-flux in rat brain membrane vesicles. Life Sci. 43: 1761-1771, 1988.

24. Cook, P.J.,Flanagan, R. and James, I.M.: Diazepam tolerance: effect of age, regular sedation, and alcohol. Br. Med. J. 289: 351-353, 1984.

25. Cook, P.J.: Benzodiazepine hypnotics in the elderly. Acta Psychiatr. Scand. 74: 149-158, 1986.

26. Crooks, J. and Stevenson, I.H.: Drug response in the elderly -sensitivity and pharmacokinetic considerations. Age and Ageing 10: 73-80, 1981.

27. Crooks, J.: Aging and drug disposition - pharmacodynamics. J. Chron. Dis. 36: 85-90, 1983.

28. De Blasi, A., Cotecchia, S. and Mennini, T.: Selective changes of receptor binding in brain regions of aged rats. Life Sci. 31: 335-340, 1982.

29. Desbarats-Schonbaum, M.-L. and Birmingham, M.K.: Effects of age of Krebs cycle metabolites on the inhibition of mouse brain respiration by pentobarbital. J. Gerontol. 14: 284-287, 1959.

30. Dingemanse, J., Danhof, M. and Breimer, D.D.: Pharmacokinetic-pharmacodynamic modelling of CNS drug effects: An overview. Pharmacol. Ther. 38: 1-52, 1988.

31. Drayer, D.E.: Pharmacodynamic and pharmacokinetic differences between drug enantiomers in humans: an overview. Clin. Pharmacol. Ther.40: 125-133, 1986.

32. Dundee, J.W.: The influence of body weight, sex and age on the dosage of thiopentone. Br. J. Anaesth. 26: 164-173, 1954.

33. Dundee, J.W., Robinson, F.P., McCollum, J.S.C. and Patterson, C.C.: Sensitivity to propofol in the elderly. Anaesthesia 41: 482-485, 1986.

34. Dumas, C., Loi, C.-M. and Cusack, B.J.: Hepatic drug metabolism and aging. Clin. Pharmacokin. 19: 359-389, 1990.

35. Duvaldestin, R., Saada, J., Berger, J.L., D'Hollander, A. and Desmonts, J.M.: Pharmacokinetics, pharmacodynamics, and dose response relationships of pancuronium in control and elderly subjects. Anesthesiol. 56: 36-40, 1982.

36. Erdö, S.L. and Wolff, J.R.: Age-related loss of TBPS binding to the GABA receptor coupled chloride ionophore in rat cerebral cortex. J. Neurochem. 53: 649-651, 1989.

37. Everitt, D.E. and Avorn, J.: Drug prescribing for the elderly. Arch. Intern. Med. 146: 2393-2400, 1986.

38. Forster, M.J., Retz, K.C., Popper, M.D. and Lal, H.: Age-dependent enhancement of diazepam sensitivity is accelerated in New Zealand Black Mice. Life Sci. 38: 1433-1439, 1986.

39. Friedel, R.O.: Effects of age on the pharmacology of tricyclic antidepressants. in: Age and the pharmacology of psychoactive drugs. A. Raskin, D.S. Robinson, J. Levine, eds., Elsevier North Holland, Inc., Amsterdam, 1981, pp 125-131.

40. Giles, H.G., Macleod, S.M., Wright, J.R. and Sellers, E.M.: Influence of age and previous use on diazepam dosage required for endoscopy. CMA Journal 118: 513-514, 1978.

41. Gordon, M. and Preiksatis, H.G.: Drugs and the aging brain. Geriatrics 43: 69-78, 1988.

42. Govoni, S., Memo, M., Saiani, L., Spano, P.F. and Trabucchi, M.: Impairment of brain neurotransmitter receptors in aged rats. Mech. Ageing Dev. 12: 39-46, 1980.

43. Greenblatt, D.J., Allen, M.D. and Shader, R.I.: Toxicity of high-dose flurazepam in the elderly. Clin. Pharmacol. Ther. 21: 355-361, 1977.

44. Greenblatt, D.J. and Allen, M.D.: Toxicity of nitrazepam in the elderly: a report from the Boston collaborative drug surveillance program. Br. J. clin. Pharmacol. 5: 407-413, 1978.

45. Greenblatt, D.J., Divoll, M., Harmatz, J.S., MacLaughlin, D.S. and Shader, R.I.: Kinetics and clinical effects of flurazepam in young and elderly noninsomniacs. Clin. Pharmacol. Ther. 30: 475-486, 1981.

46. Greenblatt, D.J., Sellers, E.M., Shader, R.I.: Drug disposition in old age. New Engl. J. Med. 306: 1081-1088, 1982.

47. Greenblatt, D.J., Abernethy, D.R., Locniskar, A., Harmatz, J.S., Limjuco, R.A. and Shader, R.I.: Effect of age, gender, and obesity on midazolam kinetics. Anesthesiol. 61: 27-35, 1984.

48. Greenblatt, D.J., Harmatz, J.S., Shapiro, L., Engelhardt N., Gouthro, T.A. and Shader, R.I.: Sensitivity to triazolam in the elderly. New Engl. J. Med. 24: 1691-1698, 1991.

49. Groen, C.: The influence of ageing on P450-mediated drug metabolism. Ph.D. Thesis. University of Leiden, 1991.

50. Guthrie, S., Cooper, R.L., Thurman R. and Linnoila, M.: Pharmacodynamics and pharmacokinetics of ethanol, diazepam and pentobarbital in young and aged rats. Pharmacol and Toxicol. 61:308-312, 1987.

51. Haaijer-Ruskamp, F.M. and Dingemans, C.A.J.: Wat krijgen ouderen aan geneesmiddelen thuis en in het verzorgingstehuis? Geriatrie-informatorium juli 1989, E 3020-1 - E 3020-13, 1989.

52. Haefely, W.E.: Pharmacology of the benzodiazepine receptor. Eur. Arch. Psychiat. Neurol. Sci. 238: 294-301, 1989.

53. Harik, S.I.: Some comments on the 'Effect of aging on the blood-brain barrier'. Neurobiol. Aging 9: 50-51, 1988.

54. Hauser, W.A. and Kurland, L.T.: The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. Epilepsica 16: 1-66, 1975.

55. Heusner, J.E. and Bosmann, H.B.: GABA Stimulation of ^{3}H diazepam binding in aged mice. Life Sci. 29: 971-974, 1981.

56. Hewick, D.S. and Shaw, V.: Tissue distribution of radioactivity after injection of ^{14}C nitrazepam in young and old rats. J. Pharm. Pharmacol. 30: 318-319, 1978.

57. Hewick, D.S.: Barbiturate sensitivity in ageing animals. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson, eds., The Macmillan Press Ltd., 1979, pp 211-219.

58. Hinrichs, J.V. and Ghoneim, M.M.: Diazepam, behavior and aging: increased sensitivity or lower baseline performance? Psychopharmacol. 92: 100-105, 1987.

59. Ho, P.C. and Triggs, E.J.: Drug therapy in the elderly. Aust. NZ. J. Med. 14: 179-190, 1984.

60. Holden, C.: Changing US demography. Science 248: 307, 1990.

61. Homer, T.D. and Stanski, D.R.: The effect of increasing age on thiopental disposition and anaesthetic requirement. Anesthesiol. 62: 714-724, 1985.

62. Ito, Y., Ho, I.K. and Hoskins, B.: Cerebellar GABA and benzodiazepine receptor characteristics in young and aged mice. Brain. Res. Bull. 21: 251-255, 1988.

63. Jones, T.W. and Beaney, J.: The effect of age and pentobarbitone tolerance on pentobarbitone depression of calcium-45 uptake by mouse brain synaptosomes. Mech. Ageing Dev. 14: 417-426, 1980.

64. Kaiko, R.F.: Age and morphine analgesia in cancer patients with postoperative pain. Clin. Pharmacol. Ther. 28: 823-826, 1980.

65. Kaiko, R.F., Wallenstein, S.L., Rogers, A.G., Grabinski, P.Y. and Houde, R.W.: Narcotics in the elderly. Med. Clin. North America 66: 1079-1089, 1982.

66. Kanto, J, Kangas, L., Aaltonen, L. and Hilke, H.: Effect of age on the pharmacokinetics and sedative effect of flunitrazepam. Int. J. Clin. Pharmacol. Ther. Toxicol. 19: 400-404, 1981.

67. Kato, R.: Drug metabolism under pathological and abnormal physiological states in animals and man. Xenobiotica 7: 25-92, 1977.

68. Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: Increased anticonvulsant effect of phenobarbital with age in mice - a possible pharmacological index for brain aging. Life Sci. 37: 1451-1460, 1985.

69. Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ahta, M. and Masuda, Y.: Age related increased threshold for electroshock seizure in BDF1 mice. Life Sci. 36, 657-662, 1985a.

70. Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: The effect of age on the adaptation of the brain to the anticonvulsant effect of phenobarbital in mice. Life Sci. 39: 483-491, 1986.

71. Kitani, K., Sato, Y., Kanai, S., Ohta, M., Nokubo, M. and Masuda, Y.: The neurotoxicity of phenobarbital and its effect in preventing pentylenetetrazole-induced maximal seizure in aging mice. Arch. Geront. Geriatr. 7: 261-271, 1988.

72. Kitani, K., Klotz, U., Kanai, S., Sato, Y., Ohta, M. and Nokubo, M.: Age-related differences in the coordination disturbance and anticonvulsant effect of oxazepam in mice. Arch. Geront. Geriatr. 9: 31-43, 1989.

73. Klotz, U. and Brückel, K.W.: Pharmacokinetics and pharmacodynamics in the elderly. in: Liver and Aging. K. Kitani, ed., Elsevier Biomedical Press, Amsterdam New York Oxford, 1982, pp 287-299.

74. Klotz, U.: Age-dependent actions of benzodiazepines. in: Drugs and Aging. D. Platt, ed., Springer - Verlag Berlin Heidelberg, 1986, pp 131-139.

75. Kochman, R.L. and Sepulveda, C.K.: Aging does not alter the sensitivity of benzodiazepine receptors to GABA modulation. Neurobiol. Aging 7: 363-365, 1986.

76. Komiskey, H.L., Cook, T.M., Lin, C.F. and Hayton W.L.: Impairment of learning or memory in the mature and old rat by diazepam. Psychopharmacol. 73: 304-305, 1981.

77. Komiskey, H.L. and MacFarlan, M.F.: Aging: Effect on neuronal and non-neuronal

benzodiazepine binding sites. Neurochem. Res. 8: 1135-1142, 1983.

78. Komiskey, H.L.: Aging: Effect on ex-vivo benzodiazepine binding after a diazepam injection. Neurochem. Res. 12: 745-750, 1987.

79. Komiskey, H.L., Buck, M.A., Mundinger, K.L., McSweeney, F.K., Farmer-Dougan, V. A. and Dougan, J.D.: Effect of aging on anticonflict and CNS depressant activity of diazepam in rats. Psychopharmacol. 13: 443-448, 1987.

80. Komiskey, H.L., Raemont, L.H. and Mundinger, K.L.: Aging: modulation of $GABA_A$ binding sites by ethanol and diazepam. Brain Res. 458: 37-44, 1988.

81. Komiskey, H.L., Rahman, A., Mundinger, K.L.: Aging: changes in a passive-avoidance response with brain levels of temazepam. Pharmacol. Bioch. Behav. 31: 611-615, 1989.

82. Kortilla, K., Saarnivaara, L., Tarkkanen, J., Himberg, J.J. and Hytönen, M.: Effect of age on amnesia and sedation induced by flunitrazepam during local anaesthesia for bronchoscopy. Br. J. Anaesth. 50: 1211-1218, 1978.

83. Lamy, P.P.: Comparative pharmacokinetic changes and drug therapy in an older population. J. Am. Geriatr. Soc. 30: 511-519, 1982.

84. Lamy, P.P.: Age-associated pharmacodynamic changes. Meth. Find. Exp. Clin. Pharmacol. 9: 153-160, 1987.

85. Lippa, A.S., Critchett, D.J., Ehlert, F., Yamamura, H.I., Enna, S.J. and Bartus, R.T.: Age-related alterations in neurotransmitter receptors: An electrophysiological and biochemical analysis. Neurobiol. Aging 2: 3-8, 1981.

86. Maggi, A., Schmidt, M.J., Ghetti, B. and Enna, S.J.: Effect of aging on neurotransmitter receptor binding in rat and human brain. Life Sci. 24: 367-374, 1979.

87. McMahon, T.F., Weiner, M., Lesko, L. and Emm, T.: Effects of age on antidepressant kinetics and memory in Fischer 344 rats. Pharmacol. Biochem. Behav. 26: 313-320, 1987.

88. Memo, M., Spano, P.F., Trabucchi, M.: Brain benzodiazepine receptor changes during ageing. J. Pharm. Pharmacol. 33: 64, 1980.

89. Meyers, M.B. and Komiskey, H.L.: Aging: Effect on the interaction of ethanol and pentobarbital with the benzodiazepine-GABA receptor-ionophore complex. Brain Res. 343: 262-267, 1985.

90. Miller R.: Anesthesia for the aged: Some pharmacokinetic and pharmacodynamic considerations. The Mount Sinai J. Med. 54: 305-307, 1987.

91. Montamat, S.C., Cusack, B.J. and Vestal R.E.: Management of drug therapy in the

elderly. New Engl. J. Med. 321: 303-309, 1989.

92. Mooradian, A.D.: Effect of aging on the blood-brain barrier. Neurobiol. Aging 9: 31-39, 1988.

93. Muravchick, S.: Effect of age and premedication on thiopental sleep dose. Anesthesiol. 61: 333-336, 1984.

94. Murray, T.G., Chiang, S.T., Hoepke, H.H., Walker, B.R.: Renal disease, age, and oxazepam kinetics. Clin. Pharmacol. Ther. 30: 805-809, 1981.

95. Niles, L.P., Pulpido, O.M. and Pickering, D.S.: Age-related changes in GABA and benzodiazepine receptor binding in rat brain are influenced by sampling time. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 12: 337-344, 1988.

96. Nokubo, M., Kitani, K., Ohta, Kanai, S., Sato, Y and Masuda, Y.: Age-dependent increase in the threshold for pentylenetetrazole induced maximal seizure in mice. Life Sci. 38: 1991-2007, 1986.

97. O'Hara, D.A., Fragen, R.J. and Shanks, C.A.: The effects of age on the dose-response curves for vecuronium in adults. Anesthesiol. 63: 542-544, 1985.

98. Ouslander, J.G.: Drug therapy in the elderly. Ann. Int. Med. 95: 711-722, 1981.

99. Pardon, I.S. and Jones, T.W.G.: Barbiturate pharmacokinetics in ageing tolerant and non-tolerant mice. in: Liver and Aging. K. Kitani, ed., Elsevier/North-Holland Biomedical Press, Amsterdam New York Oxford, 1978, pp 301-311.

100. Pardridge, W.M.: Does the brain's gatekeeper falter in aging? Neurobiol. Aging 9: 44-46, 1988.

101. Pedigo, N.W., Schoemaker, H., Morelli, M., McDougal, J.N., Malick, J.B., Burks, T.F. and Yamamura, H.I.: Benzodiazepine receptor binding in young, mature and senescent rat brain and kidney. Neurobiol. Aging 2: 83-88, 1981.

102. Petty, W.C. and Karler, R.: The influence of aging on the activity of anticonvulsant drugs. J. Pharmacol. Exp. Ther. 150: 443-448, 1965.

103. Pomara, N., Stanley, B., Block, R., Guido, J., Russ, D., Berchou, R., Stanley, M., Greenblatt, D.J., Newton, R.E. and Gershon, S.: Adverse effects of single therapeutic doses of diazepam on performance in normal geriatric patients: Relationship to plasma concentrations. Psychopharmacol. 84: 342-346, 1984.

104. Pomara, N., Stanley, B., Block, R., Berchou, R.C., Stanley, M., Greenblatt, D.J., Newton, R.E. and Gershon, S.: Increased sensitivity of the elderly to the central depressant effects of diazepam. J. Clin. Psychiat. 46: 185-187, 1985.

105. Pomara, N., Deptula, D., Medel, M., Block, R.I. and Greenblatt, D.J.: Effects of diazepam on recall memory: Relationship to aging, dose, and duration of treatment. Psychopharmacol. Bull. 25: 144-148, 1989.

106. Pucino, F., Beck, C.L., Seifert, R.L., Strommen, G.L., Sheldon, P.A. and Silbergleit, I.L.: Pharmacogeriatrics. Pharmacother. 5: 314-326, 1985.

107. Rahman A., Komiskey, H.L., Hayton, W.L. and Weisenburger, W.P.: Aging: changes in distribution of diazepam and metabolites in the rat. Drug Metab. Disp. 14: 299-302, 1986.

108. Rapoport, S.I., Ohno, K. and Pettigrew, K.D.: Blood-brain barrier permeability in senescent rats. J. Gerontol. 34: 162-169, 1979.

109. Reeves, P.M. and Schweizer, M.P.: Aging, diazepam exposure and benzodiazepine receptors in rat cortex. Brain Res. 70: 376-379, 1983.

110. Reidenberg, M.M., Levy, M., Warner, H., Couthino, C.B., Schwartz, M.A., Yu, G. and Cheripko, J.: Relationship between diazepam dose, plasma level, age, and central nervous system depression. Clin. Pharmacol. Ther. 23: 371-374, 1978.

111. Ritschel, W.A.: Pharmacokinetic changes in the elderly. Meth. Find. Exp. Clin. Pharmacol. 9: 161-166, 1987.

112. Roberts, J. and Turner, N.: Age and diet effects on drug action. Pharmacol. Ther. 37: 111-149, 1988.

113. Roberts, J. and Turner, N.: Pharmacodynamic basis for altered drug action in the elderly. Clinics in Geriatr. Med. 4: 127-149, 1988a.

114. Roehrs, T., Zorick, F., Wittig, R. and Roth, T.: Efficacy of a reduced triazolam dose in elderly insomniacs. Neurobiol. Aging 6: 293-296, 1985.

115. Rupp, S.M., Castagnoli, K.P., Fisher, D.M. and Miller, R.D.: Pancuronium and vecuronium pharmacokinetics and pharmacodynamics in younger and elderly adults. Anesthesiol. 67: 45-49, 1987.

116. Salzman, C.: Stimulants in the elderly. in: Age and the pharmacology of psychoactive drugs. A. Raskin, D.S. Robinson, J. Levine, eds., Elsevier North Holland, Inc., Amsterdam, 1981, pp 171-180.

117. Salzman, C.: A primer of geriatric psychopharmacology. Am. J. Psychiat. 139: 67-74, 1982.

118. Saunders, D.R., Paolino, R.M., Bousquet, W.F. and Miya, T.S.: Age-related responsiveness of the rat to drugs affecting the central nervous system. Proc. Soc. Exp. Biol. Med. 147: 593-595, 1974.

119. Schüttler, J., Stanski, D.R., White, P.F., Trevor, A.J., Horai, Y., Verotta, D. and Sheiner, L.B.: Pharmacodynamic modeling of the EEG effects of ketamine and its enantiomers in man. J. Pharmacokin. Biopharm. 15: 241-253, 1987.

120. Schwartz, R.D.: The $GABA_A$ receptor-gated ion channel: biochemical and pharmacological studies of structure and function. Biochem. Pharmacol. 37: 3369-3375, 1988.

121. Scott, P.J.W.: Review: Effect of age on pharmacodynamics in man. J. Clin. Exp. Gerontol. 4: 205-226, 1982.

122. Scott, J.C. and Stanski, D.R.: Decreased fentanyl and alfentanil dose requirements with age. - A simultaneous pharmacokinetic and pharmacodynamic evaluation. J. Pharmacol. Exp. Ther. 240: 159-166, 1987.

123. Servin, F., Enriquez, I.,Fournet, M., Failler, J.M., Farinotti, R. and Desmonts, J-M.: Pharmacokinetics of midazolam used as an intravenous induction agent for patients over 80 years of age. Eur. J. Anaesth. 4: 1-7, 1987.

124. Shock, N.W.: Physiological aspects of aging in man. Ann. Rev. Physiol. 23: 97-122, 1961.

125. Shock, N.W.: Aging of physiological systems. J. Chron. Dis. 36: 137-142, 1983.

126. Smith, D.S., Orkin, F.K., Gardner, S.M. and Zakeosian, G.: Prolonged sedation in the elderly after intraoperative atropine administration. Anesthesiol. 51: 348-349, 1979.

127. Stanski, D.R. and Maitre, P.O.: Population pharmacokinetics and pharmacodynamics of thiopental: The effect of age revisited. Anesthesiol. 72: 412-422, 1990.

128. Stevenson, I.H., Hockings, N.F. and Swift, C.G.: Pharmacokinetics and pharmacodynamics of single doses of hypnotic drugs in healthy elderly subjects. in: Liver and Aging. K. Kitani, ed., Elsevier Biomedical Press, Amsterdam New York Oxford, 1982, pp 317-330.

129. Stevenson, I.H.: Drugs for the elderly. in: Proc. Sec. World Conference on Clin. Pharmacol. Ther.. L. Lemberger and M.M. Reidenberg, eds., Am. Soc. Pharmacol. Exp. Ther., Bethesda, 1984, pp 64-73.

130. Stevenson, I.H. and Hosie, J.: Pharmacokinetics in the elderly. in: Therapeutics in the elderly. K. O'Malley and J.L. Waddington, eds., Elseviers Science Publishers B.V. (Biomedical Division), Amsterdam, 1985, pp 35-41.

131. Streicher, E. and Garbus, J.: The effect of age and sex on duration of hexobarbital anaesthesia in rats. J. Gerontol. 10: 441-444, 1955.

132. Swift, C.G., Haythorne, J.M., Clarke, P. and Stevenson, I.H.: The effect of ageing on

measured responses to single doses of oral temazepam. Br. J. clin. Pharmacol. 11, 413P-414P, 1981.

133. Swift, C.G., Swift, M.R., Hamley, J., Stevenson, I.H. and Crooks, J.: Side-effect 'tolerance' in elderly long-term recipients of benzodiazepine hypnotics. Age Ageing 13: 335-343, 1984.

134. Swift, C.G., Swift, M.R., Ankier, S.I., Pidgen, A. and Robinson, J.: Single dose pharmacokinetics and pharmacodynamics of oral loprazolam in the elderly. Br. J. clin. Pharmacol. 20, 119-128, 1985.

135. Swift, C.G., Ewen, J.M., Clarke, P. and Stevenson, I.H.: Responsiveness to oral diazepam in the elderly: relationship to total and free plasma concentrations. Br. J. clin. Pharmacol. 20, 111-118, 1985a.

136. Swift, C.G.: Benzodiazepine pharmacodynamics in the elderly. in: Therapeutics in the elderly. K. O'Malley and J.L. Waddington, eds., Elseviers Science Publishers B.V. (Biomedical Division), Amsterdam, 1985b, pp. 77-90.

137. Tregaskis, B.F. and Stevenson, I.H.: Pharmacokinetics in old age. Br. Med. Bull. 46: 9-21, 1990.

138. Tsang, C.C., Speeg, K.V. and Wilkinson, G.R.: Aging and benzodiazepine binding in rat cerebral cortex. Life Sci. 30: 343-346, 1982.

139. Tsujimoto, G., Hashimoto, K. and Hoffman, B.B.: Pharmacokinetic and pharmacodynamic principles of drug therapy in old age. Part 1. Int. J. Clin. Pharmacol. Ther. Toxicol. 27: 13-26, 1989.

140. Tsujimoto, G., Hashimoto, K. and Hoffman, B.B.: Pharmacokinetic and pharmacodynamic principles of drug therapy in old age. Part 2. Int. J. Clin. Pharmacol. Ther. Toxicol. 27: 102-116, 1989a.

141. Van Bezooijen, C.F.A.: Bijverschijnselen van geneesmiddelen bij ouderen. T. Gerontol. Geriatr. 16: 191-198, 1985.

142. Van Bezooijen, C.F.A.: Changes in pharmacokinetics with age in man. A review. in: Liver, drugs and aging. C.F.A. van Bezooijen, F. Miglio and D.L. Knook, eds., EURAGE, Rijswijk, 1986, pp 25-32.

143. Van Bezooijen, C.F.A.: Bijwerkingen en ziekenhuisopname ten gevolge van geneesmiddelengebruik bij oudere patiënten. Geriatrie-informatorium maart 1989: E 3035-1 - E 3035-19, 1989.

144. Veering, B. Th., Burm, A.G.L., van Kleef, J.W., Hennis, P.J. and Spierdijk, J.: Epidural anesthesia with bupivacaine: Effects of age on neural blockade and pharmacokinetics. Anesth. Analg. 66: 589-593, 1987.

145. Veering, B. Th., Burm, A.G.L., van Kleef, J.W., Hennis, P.J. and Spierdijk, J.: Spinal anesthesia with glucose-free bupivacaine: Effects of age on neural blockade and pharmacokinetics. Anesth. Analg. 66: 965-970, 1987a.

146. Virtanen, R., Kanto, J., Iisalo, E., Salo, M. and Sjövall, S.: Pharmacokinetic studies on atropine with special reference to age. Acta Anaesth. Scand. 26: 297-300, 1982.

147. Wallace, S.M. and Verbeeck, R.K.: Plasma protein binding of drugs in the elderly. Clin. Pharmacokin. 12: 41-72, 1987.

148. Wanwimolruk, S. and Levy, G.: Effect of age on the pharmacodynamics of phenobarbital and ethanol in rats. J. Pharm. Sci. 76: 503-507, 1987.

149. Young, W.L., Matteo, R.S. and Ornstein, E.: Duration of action of neostigmine and pyridostigmine in the elderly. Anesth. Analg. 67: 775-778, 1988.

Chapter 2

METHODOLOGY OF STUDIES ON AGE-RELATED CHANGES IN *IN VIVO* PHARMACODYNAMICS IN RATS

1. Introduction

As was concluded in chapter 1, more extensive and rigorously designed pharmacodynamic studies are needed to investigate the nature and degree of changes in the sensitivity of the brain to barbiturates and benzodiazepines during ageing. For this purpose *in vivo* studies are a prerequisite, since the pharmacological effect of these drugs is established via a cascade of several processes in the body. If an age-related change in brain sensitivity is found, in vitro studies are indicated to investigate the mechanism behind this change (Barnhill et al., 1990). Because of this combination of *in vivo* and *in vitro* studies, the use of an animal model of ageing is indispensable. Extra advantages are the possibility for more invasive *in vivo* investigations in animals (thereby offering the possibility for a more rigorous differentiation between pharmacokinetics and pharmacodynamics) and the lower variability compared to that in man, because of the possibility to use inbred rat strains and to minimize differences in environmental factors (Scott, 1982). Moreover, a rigorous differentiation between ageing *per se* and concomitant pathology is in principle possible.

2. Gerontological studies

2.1. The animal model of ageing employed

Rats are frequently used in ageing research, because of their relatively short life spans, ease of handling and the relatively low costs of breeding and

maintenance. The use of strict breeding conditions and careful monitoring of environmental conditions have reduced infectious diseases and improved the health status of the animals, which has contributed to the increase of animal life span (Hollander et al., 1990).
One prerequisite of the rat strain used as an animal model of ageing is the availability of data on survival curves, as a reference for the ages of the rats. Since ageing is associated with a growing incidence of disease (Burek, 1978; Zurcher and Hollander, 1982; Brody and Schneider, 1986; Hollander et al., 1990), it is very important to consider the health status of the subjects under investigation when performing research on age-related changes, in order to be able to differentiate between true age-related changes versus disease-related changes (Pucino et al., 1985; Williams, 1987; Ligthart, 1989). Therefore, an animal strain is needed which exhibits a relatively low incidence of age-related pathology. Moreover, this pathology has to be well characterized (Hollander, 1979). In the experiments described in this thesis the BN/BiRij rat was used. The age-related pathology of this strain has been extensively investigated (Burek, 1978).
Although a rat strain with a low incidence of pathology is used, it is nevertheless very important to evaluate the health status of the individual animals used in gerontological research by measuring clinical biochemical indices and examining important organs microscopically.

2.2. Cross-sectional versus longitudinal study design

In principle, two different types of study design can be chosen in gerontological research (Rowe, 1977). For every new study, it has to be considered which study design is the most appropriate. In a cross-sectional design, groups of various ages are investigated (ideally at one time point) and differences between the groups are evaluated. In longitudinal studies, serial measurements are obtained on one group of subjects at specified ages during their lives. If the intraindividual variability is lower than the interindividual variability in the parameter, which is being studied during ageing, the longitudinal design may be more powerful to detect age-related differences. In addition, in longitudinal studies the selection of the biologically superior survivors in the age groups over 50% survival does not lead to a misinterpretation of the results, since the animals are studied throughout their whole lifetime (Rowe, 1977).
A pitfall of cross-sectional studies is, that differences found between the age

groups can be caused by cohort and interindividual differences instead of being related to the age of the subjects (Schlettwein-Gsell, 1970; Lesser et al., 1973; Curcio et al., 1984; Mos and Hollander, 1987). This uncertainty is overcome by applying a longitudinal design. One drawback of longitudinal studies is the sensitivity to alterations in methods used over the years, including change of investigators during the course of one experiment. Another drawback is the problem, that the second experiment in one animal can be influenced by changes in the animal due to the first experiment ('carry-over' effects). Moreover, it is not possible to perform an extensive pathological evaluation (post-mortem tissue examination) in every animal directly after the investigations, which is important to be able to differentiate between age-related changes and disease-related changes. Also from a practical point of view, application of a longitudinal design in pharmacodynamic studies in animals is not always possible, because cannulas or electrodes implanted necessary for pharmacodynamic investigations will not remain intact over the lifetime of the animals.

2.3. Age range included in the investigations

In studies in humans the ages of the oldest subjects do not always exceed the age of 65 years, which is below the median life expectancy (Bickford-Wimer et al., 1987). Especially in investigations in animals the ages of the subjects studied are below the age of 50% survival. In most reports in literature the ages are given without data on survival curves, which are indispensable for an unambiguous interpretation of the ages of animals (Hollander, 1979). The most important pharmacological changes in humans often take place above the age of 60 years (Christensen and Andreasen, 1978; Kortilla et al., 1978; Kanto et al., 1981; Dundee et al., 1986; Bell et al., 1987; Roberts and Turner, 1988). It is necessary therefore, to include the very old animals in gerontological studies (ages around 50 and 90% survival). Because it is important to investigate at which age an age-related change starts to appear, one should not only study one group of young and one group of old rats, but also some ages in between. In the studies, presented in this thesis, four to seven age groups, aged between 3 and 37 months, were used.

3. *In vivo* pharmacodynamic studies of anaesthetics and anticonvulsants

3.1. The measure of the pharmacological effect

In order to be able to investigate the relationship between the concentration of a drug and the pharmacological effect, the availability of a quantitative measure of the pharmacological effect is a prerequisite. Ideally, such measures are continuous, sensitive, objective, reproducible within and between subjects, obtainable both in experimental animals and in man and meaningful with regard to a relevant clinical effect (Dingemanse et al., 1988; Danhof, 1989). Since *in vivo* pharmacodynamics is still a developing branch of science, the effect measures available at the moment for quantitating the effects of anaesthetics and anticonvulsants do not (yet) meet all criteria mentioned.

In the experiments described in this thesis three different techniques to quantitate the pharmacological effect of CNS active drugs were applied. The technique of onset or offset of loss of righting reflex (LRR) is used as a measure of the anaesthetic effect of barbiturates. During continuous infusion or after bolus administration of the drug, respectively, the point of disappearance (in case of onset of LRR) or reappearance (in case of offset of LRR) of a reflex reaction of the animal upon stimulation of the tail is determined, together with the concentration needed to attain this effect (Danhof and Levy, 1984; Dingemanse et al., 1988a,b). This means that only one point of the concentration vs. effect relationship can be determined for each animal.

The second technique uses the drug-induced changes in the electroencephalogram (EEG) as a measure the pharmacological effect. For benzodiazepines it was shown that the drug-induced changes in the EEG reflect the GABAergic inhibition component of the pharmacological effect (Mandema et al., 1991). The EEG is registered continuously after administration of the drug until the baseline level is reached again. The technique of aperiodic analysis is applied to determine amplitude and frequency of the EEG signal on a wave by wave basis (Gregory and Pettus, 1986). The gradual changes in amplitude and frequency with changing concentration are used as a measure of the effect. In this way complete concentration vs. EEG effect relationships can be determined in individual

animals (Mandema et al., 1990).
The anticonvulsant effect was quantitated using the third technique, namely a direct cortical stimulation method (Voskuyl et al., 1989). In this method, bipolar pulse trains of increasing amplitude, directed to the frontoparietal cortex through permanently implanted electrodes, are applied as a convulsant stimulus, and the threshold to induce localized seizure activity is determined. After bolus administration or during continuous administration of an anticonvulsant the threshold is determined repeatedly and the elevation of the threshold above the baseline level is used as a measure of the anticonvulsant effect. Because repeated measurement of the threshold is possible, complete concentration vs. effect relationships can be determined in individual animals (Dingemanse et al., 1990).

3.2. Pharmacodynamic model

Drug concentration vs. pharmacological effect profiles are generally examined according to mathematical pharmacological models (Holford and Sheiner, 1981, 1982; Dingemanse et al., 1988; Danhof, 1989). In the investigations described in this thesis the sigmoidal E_{max} model has been applied to characterize the concentration vs. EEG effect relationships of heptabarbital and midazolam. In this model the relationship between concentration and pharmacological effect is described by the following equation:

$$E = E_0 + \frac{E_{max}.C^n}{C^n + EC_{50}{}^n}$$

where E is the observed effect, E_0 is the baseline effect value, E_{max} is the maximum attainable effect, C is the hypothetical effect compartment concentration, EC_{50} the effect compartment concentration causing half the maximum effect and n is a parameter that determines the steepness of the curve. The hypothetical effect compartment concentration is calculated using the plasma concentration vs. pharmacological effect profile (this will be discussed in more detail further on in this chapter).

3.3. Pharmacokinetic complications in pharmacodynamic studies

The results of pharmacodynamic studies *in vivo* can be influenced by several pharmacokinetic factors, which obscure the true underlying concentration vs. effect relationship (Danhof et al., in press). In studies on pharmacodynamics

and ageing, these pharmacokinetic factors can also display age-related changes, which leads to a misinterpretation of the influence of ageing on the pharmacodynamics. When a drug is administered as a mixture of enantiomers, which differ in pharmacological activity, possible age-related changes in the disposition of these enantiomers (Chandler et al., 1988) give rise to problems on the interpretation of the concentration vs. effect profiles. The same holds for age-related changes in the formation of (inter)active metabolites (Durnas et al., 1990) and in the development of acute tolerance (Cook et al., 1983), which can have both a pharmacokinetic and a pharmacodynamic background. A fourth pharmacokinetic complication is related to the fact that in pharmacodynamics the relationship between the concentration at the site of action and the pharmacological effect is studied. Since it is impossible to directly measure the concentrations at the site of action, concentrations are mostly measured in a more accessible compartment, the blood. Age-related changes in the distribution of a drug between blood and site of action (Hewick and Shaw, 1978; Pardon and Jones, 1978; Rahman et al., 1986; Kitani et al., 1989) can lead to misinterpretation of the blood concentration vs. effect curves.

Several direct or indirect techniques are available to take these pharmacokinetic complicating factors into consideration. In order to circumvent the problems related to the use of chiral compounds, purified enantiomers can be applied, although one should be aware of the possible metabolic inversion in the body (Vermeulen and Breimer, 1983). If available, another model compound might be chosen which is not optically active.

The contribution of (inter)active metabolites to the pharmacological effect of a drug can be studied by direct administration of the metabolite and analyzing the effects, by analysis of the pharmacological effect following different routes and modes of administration of the parent compound and by utilizing radioreceptor assays, by which plasma concentrations of the active metabolite(s) and the parent compound together are measured after administration of the parent compound (Danhof and Levy, 1984; Klockowski and Levy, 1988; Dingemanse et al., 1988, 1988b, 1988c, 1989; Mandema et al., 1991a; Danhof et al., in press). If an (inter)active metabolite is formed, the role of this metabolite should be determined.

Indications for the occurrence of acute tolerance can be obtained on analyzing the pharmacological effect of a drug following different modes of administration (Dingemanse et al., 1988b), although one should be aware of

the fact that a metabolite with antagonistic properties gives rise to results comparable to those caused by tolerance (Holford and Sheiner, 1981). It is possible to describe a concentration vs. effect profile showing the occurrence of tolerance development using mathematical pharmacodynamic models (Porchet et al., 1988).
In order to determine the relationship between the concentration of the drug at the site of action and the pharmacological effect one may try to identify a body compartment (from which samples can be taken in in vivo experiments) which is in equilibrium with the site of action, although other compartments are not (Danhof and Levy, 1984). Another possibility is the application of the concept of the effect compartment model (Sheiner et al., 1979; Holford and Sheiner, 1981a), in which a hypothetical effect compartment is introduced which receives its input from the central compartment by a first-order process. From the blood (or plasma or serum) concentration vs. pharmacological effect curves the rate constant describing the rate of equilibration between the blood and the effect site may be calculated, with subsequent estimation of the concentrations in the effect compartment.

4. Conclusion

For studying the effect of ageing on *in vivo* pharmacodynamics, an animal strain is required of which survival curves and age-related pathology data are known. The old animals should show a relatively low degree of pathology and the health status of all animals included in pharmacodynamic investigations should be carefully assessed. Animals aged around 50 and 10% survival should at least be included. For every study, it has to be considered, whether a cross-sectional or a longitudinal study design is the most appropriate.
Using a suitable parameter for the quantitation of the pharmacological effect, preferably complete concentration vs. effect profiles should be constructed and mathematically described by a pharmacodynamic model. Complicating factors, like the application of a chiral compound, the formation of (inter)active metabolites, the development of acute tolerance and the distribution between the sampling site and the site of action should be taken into account.

5. References

Barnhill, J.G., Greenblatt, D.J., Miller, L.G., Gaver, A., Hartmatz, J.S. and Shader, R.I.: Kinetic and dynamic components of increased benzodiazepine sensitivity in aging animals. J. Pharmacol. Exp. Ther. 253: 1153-1161, 1990.

Bell, G.D., Spickett, G.P., Reeve, P.A., Morden, A. and Logan, R.F.A.: Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. Br. J. clin. Pharmacol. 23: 241-244, 1987.

Bickford-Wimer, P.C., Parfitt, K., Hoffer, B.J. and Freedman, R.: Desipramine and noradrenergic neurotransmission in aging: failure to respond in aged laboratory animals. Neuropharmacol. 26: 597-606, 1987.

Brody, J.A. and Schneider, E.L.: Diseases and disorders of aging: an hypothesis. J. Chron. Dis. 39: 871-876, 1986.

Burek, J.D.: Pathology of ageing rats. A morphological and experimental study of the age-associated lesions in aging BN/Bi, Wag/Rij and (WAG x BN) F_1 rats. CRC-Press Inc., West Palm Beach, Florida, 1978.

Chandler, M.H.H., Scott, S.R. and Blouin, R.A.: Age-associated stereoselective alterations in hexobarbital metabolism. Clin. Pharmacol. Ther. 43: 436-441, 1988.

Christensen, J.H. and Andreasen, F.: Individual variation in response to thiopental. Acta Anaesthesiol. Scand. 22: 303-313, 1978.

Cook, P.J., Huggett, A., Graham-Pole, R., Savage, I.T. and James, I.M.: Hypnotic accumulation and hangover in elderly patients: a controlled double-blind study of temazapam and nitrazepam. Br. Med. J. 286: 100-102, 1983.

Curcio, C.A., McNelly, N.A. and Hinds, J.W.: Variation in longevity of rats: evidence for a systematic increase in lifespan over time. Exp. Aging Res. 10: 137-140, 1984.

Danhof, M.: Does variability in drug disposition explain (all) variability in drug effects? in: Topics in Pharmaceutical Sciences 1989. D.D. Breimer, K.K. Midha, D.J.A. Crommelin, eds., Amsterdam Medical Press, Noordwijk, The Netherlands, 1989, pp 573-586.

Danhof, M. and Levy, G.: Kinetics of drug action in disease states. I. Effect of infusing rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 229: 44-50, 1984.

Danhof, M., Mandema, J.W. and Stijnen, A.M.: Pharmacokinetic complexities of pharmacodynamic studies *in vivo*. in: The *in vivo* study of drug action. Principles and applications of kinetic-dynamic modelling. C.J. van Boxtel, M. Danhof and N.H.G. Holford, eds., in press.

Dingemanse, J., Danhof, M. and Breimer, D.D.: Pharmacokinetic-pharmacodynamic modelling

of CNS drug effects: An overview. Pharmacol. Ther. 38: 1-52, 1988.

Dingemanse, J., Thomassen, D., Mentink B.H. and Danhof M.: Strategy to assess the role of (inter)active metabolites in pharmacodynamic studies in-vivo: a model study with heptabarbital. J. Pharm. Pharmacol. 40: 552-557, 1988a.

Dingemanse, J., Hutson, P.H., Langemeijer, M.W.E., Curzon, G. and Danhof, M.: Application of serial sampling of cerebrospinal fluid in pharmacodynamic studies with a drug active in the CNS: Heptabarbital concentrations at onset and offset of loss of righting reflex in rats. Neuropharmacol. 27: 467-474, 1988b.

Dingemanse, J., Sollie, F.A.E., Breimer, D.D. and Danhof, M.D.: Pharmacokinetic modelling of the anticonvulsant response of oxazepam in rats using the pentylenetetrazol threshold concentration as pharmacodynamic measure. J. Pharmacokin. Biopharm. 16: 203-228, 1988c.

Dingemanse, J., van Bree, J.B.M.M. and Danhof, M.: Pharmacokinetic modeling of the anticonvulsant action of phenobarbital in rats. J. Pharmacol. Exp. Ther. 249: 601-608, 1989.

Dingemanse, J., Voskuyl, R.A., Langemeijer, M.W.E., Postel-Westra, I., Breimer, D.D., Meinardi, H. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the anticonvulsant effect of oxazepam in individual rats. Br. J. Pharmacol. 99: 53-58, 1990.

Dundee, J.W., Robinson, F.P., McCollum, J.S.C. and Patterson, C.C.: Sensitivity to propofol in the elderly. *Anaesthesia* 41: 482-485, 1986.

Durnas, C., Loi, C.-M. and Cusack, B.J.: Hepatic drug metabolism and aging. Clin. Pharmacokin. 19: 359-389, 1990.

Gregory, T.K. and Pettus, D.C.: An electroencephalographic processing algorithm specifically intended for analysis of cerebral activity. J. Clin. Monit. 2: 190-197, 1986.

Hewick, D.S. and Shaw, V.: Tissue distribution of radioactivity after injection of ^{14}C nitrazepam in young and old rats. J. Pharm. Pharmacol. 30: 318-319, 1978.

Holford, N.H.G. and Sheiner, L.B.: Understanding the dose-effect relationship: clinical application of pharmacokinetic-pharmacodynamic models. Clin. Pharmacokin. 6: 429-453, 1981.

Holford, N.H.G. and Sheiner, L.B.: Pharmacokinetic and pharmacodynamic modeling *in vivo*. CRC critical reviews in bioengineering 5: 273-322, 1981a.

Holford, N.H.G. and Sheiner, L.B.: Kinetics of pharmacologic response. Pharmacol. Ther. 16: 143-166, 1982.

Hollander, C.F.: Pathophysiology of ageing in animal models. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson, eds., The Macmillan Press Ltd., 1979, pp 15-22.

Hollander, C.F., Delort, P. and Duprat, P.: Toxicology in the older age group. in: Progress in predictive toxicology. D.B. Clayson, I.C. Munro, P. Shubik, J.A. Swenberg, eds., Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 1990, pp 113-142.

Kanto, J, Kangas, L., Aaltonen, L. and Hilke, H.: Effect of age on the pharmacokinetics and sedative effect of flunitrazepam. Int. J. Clin. Pharmacol. Ther. Toxicol. 19: 400-404, 1981.

Kitani, K., Klotz, U., Kanai, S., Sato, Y., Ohta, M. and Nokubo, M.: Age-related differences in the coordination disturbance and anticonvulsant effect of oxazepam in mice. Arch. Geront. Geriatr. 9: 31-43, 1989.

Klockowski, P.M. and Levy, G.: Kinetics of drug action in disease states. XXIV. Pharmacodynamics of diazepam and its active metabolites in rats. J. Pharmacol. Exp. Ther. 244: 912-918, 1988.

Kortilla, K., Saarnivaara, L., Tarkkanen, J., Himberg, J.J. and Hytönen, M.: Effect of age on amnesia and sedation induced by flunitrazepam during local anaesthesia for bronchoscopy. Br. J. Anaesth. 50: 1211-1218, 1978.

Lesser, G., Deutsch, T.S. and Markofsky, J.: Aging in the rat: longitudinal and cross-sectional study of body composition. Am. J. Physiol. 225, 1472-1478, 1973.

Ligthart, G.J.: The immune system in human ageing. Necessity of the assessment of health status in gerontological studies. Thesis, University of Leiden, 1989.

Mandema, J.W. and Danhof, M.: Pharmacokinetic/ pharmacodynamic modelling of the central nervous system effects of heptabarbital using aperiodic EEG analysis. J. Pharmacokin. Biopharm. 18: 459-481, 1990.

Mandema, J.W., Sansom, L.N., Dios-Vièitez, M.C., Hollander-Jansen, M. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the EEG effects of benzodiazepines. Correlation with receptor binding and anticonvulsant activity. J. Pharmacol. Exp. Ther. 257: 472-478, 1991.

Mandema, J.W., Tukker, E. and Danhof, M.: Pharmacokinetic/ pharmacodynamic modelling of EEG effects of midazolam in individual rats: influence of rate and route of administration. Br. J. Pharmacol., 102: 663-668, 1991a.

Mos, J. and Hollander, C.F.: Analysis of survival data on aging rat cohorts: Pitfalls and some practical considerations. Mech. Ageing Dev. 38: 89-105, 1987.

Pardon, I.S. and Jones, T.W.G.: Barbiturate pharmacokinetics in ageing tolerant and non-tolerant mice. in: Liver and Aging. K.Kitani, ed., Elsevier/North-Holland Biomedical Press, Amsterdam New York Oxford, 1978, pp 301-311.

Porchet, H.C., Benowitz, N.L. and Sheiner, L.B.: Pharmacodynamic model of tolerance: Application to nicotine. J. Pharmacol. Exp. Ther. 244: 231-236, 1988.

Pucino, F., Beck, C.L., Seifert, R.L., Strommen, G.L., Sheldon, P.A. and Silbergleit, I.L.: Pharmacogeriatrics. Pharmacother. 5: 314-326, 1985.

Rahman A., Komiskey, H.L., Hayton, W.L. and Weisenburger, W.P.: Aging: changes in distribution of diazepam and metabolites in the rat. Drug Metab. Disp. 14: 299-302, 1986.

Roberts, J. and Turner, N.: Pharmacodynamic basis for altered drug action in the elderly. Clinics in Geriatr. Med. 4: 127-149, 1988.

Rowe, J.W.: Clinical research on aging: strategies and directions. New Engl. J. Med. 297: 1332-1336, 1977.

Schlettwein-Gsell, D.: Survival curves of an old rat colony. Gerontologia 16: 111-115, 1970.

Scott, P.J.W.: Review: Effect of age on pharmacodynamics in man. J. Clin. Exp. Gerontol. 4: 205-226, 1982.

Sheiner, L.B., Stanski, D.R., Vozeh, S., Miller, R.D. and Ham, J.: Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. Clin. Pharmacol. Ther. 25: 358-371, 1979.

Vermeulen, N.P.E. and Breimer, D.D.: Stereoselectivity in drug and xenobiotic metabolism. in: *Stereochemistry and biological activity of drugs.* E.J. Ariëns, W. Soudijn and P.B.M.W.M. Timmermans, eds., Blackwell, Oxford, 1983, pp 33-53.

Voskuyl, R.A., Dingemanse, J. and Danhof, M.: Determination of the threshold for convulsions by direct cortical stimulation. Epilepsy Res. 3: 120-129, 1989.

Williams, T.F.: Aging or disease? Clin. Pharmacol. Ther. 42: 663-665, 1987.

Zurcher, C. and Hollander, C.F.: Multiple pathological changes in aging rat and man. Expl. Biol. Med. 7: 56-62, 1982.

Chapter 3

OBJECTIVES AND INTRODUCTION TO THE VARIOUS INVESTIGATIONS

I. General objectives

As will be already clear from chapter 1 and 2, the primary aim of the investigations described in this thesis was to study the influence of ageing on the *in vivo* pharmacodynamics of sedative and anticonvulsant drugs in rats, excluding interfering pharmacokinetic factors and the contribution of concomitant pathology. In order to be able to distinguish between changes due to ageing and changes due to (age-related) pathology, all animals were subjected to an extensive clinical biochemical/ pathological evaluation. Before the start of the experiment clinical biochemical indices for liver and kidney function and a few general indices were measured. After the experiment the animals were sacrificed and liver, kidneys, heart, lungs, brain and macroscopically visible abnormalities were evaluated by an independent pathologist. Diseased animals were excluded from the evaluation of the results or, if necessary or desirable, evaluated as a separate group.
When studying pharmacodynamics, several pharmacokinetic complicating factors (distribution to the site of action, protein binding, active metabolites, acute tolerance, stereoselective disposition of enantiomers) have to be considered. Therefore, if possible, also the pharmacokinetics were investigated in the same animals. This allowed a quantitative assessment of pharmacokinetic-pharmacodynamic relationships and thereby the pharmacodynamics could be studied unambiguously.
All studies in this thesis were performed in male BN/BiRij rats as an animal model of ageing.

II. Anaesthetic effect

Phenobarbital and heptabarbital were used as model compounds for studying age-related changes in the pharmacodynamics of the anaesthetic effect of

barbiturates (chapters 4, 5 and 6). These two barbiturates were chosen, since they are not optically active, no (inter)active metabolites are formed and no acute tolerance develops (Danhof and Levy, 1984; Dingemanse et al., 1988, 1988a). The technique of loss of righting reflex was applied as a measure of the anaesthetic effect and the concentration of the barbiturate in cerebrospinal fluid at onset (for phenobarbital) or offset (for heptabarbital) of loss of righting reflex was used as a measure of the sensitivity of the brain to these barbiturates.

The pharmacokinetics of both drugs were studied, including the relative distribution of the barbiturates between plasma (total and unbound drug concentration), brain and cerebrospinal fluid. For phenobarbital also the excretion of the main metabolite p-hydroxyphenobarbital into urine and faeces was investigated (chapter 4).

Age-related changes in pharmacokinetics and pharmacodynamics of phenobarbital were examined in a cross-sectional (chapter 4) and a 'longitudinal' study design (chapter 5), to verify whether changes found in the cross-sectional design are (partly) due to cohort differences. In the applied 'longitudinal' design, the animals were not investigated several times during their lives (as is usually done in longitudinal studies), but one group of animals, born within a period of two weeks, was reserved for the study and at five different ages one subgroup was investigated. For all other studies in this thesis only a cross-sectional design was used. Parallel to the 'longitudinal' study, also the reproducibility over a period of 2.5 years of the applied technique was investigated in young rats (chapter 5).

In the study on age-related changes in pharmacokinetics and pharmacodynamics of heptabarbital (chapter 6), a relatively high percentage of the rats displayed pathology. Therefore, the results were evaluated in two ways: considering only the healthy rats and including also the diseased rats. In addition, in the oldest rats in this study the results in the healthy rats and those in the diseased ones were compared.

III. EEG effects

The pharmacodynamics of heptabarbital were also studied during ageing using the heptabarbital-induced changes in the EEG as a measure of the pharmacological effect (chapter 7) and the results were compared with those obtained in the study in which the technique of loss of righting reflex was applied as a measure of the anaesthetic effect of heptabarbital (chapter 6).

The influence of ageing on the EEG effects and the pharmacokinetics of the benzodiazepine midazolam, which is frequently used in anaesthesia, was studied in chapter 8. Acute tolerance and (inter)active metabolites were reported not to contribute to the concentration vs. EEG effect relationship of midazolam (Mandema et al., 1991). The rationale for this study was the important age-related decrease in sedative dose requirement (about 75% between the ages of 15 and 85 years), reported in a study comprizing 800 subjects (Bell et al., 1987).

IV Anticonvulsant effects

The age-related changes in the pharmacokinetics and the anticonvulsant effect vs. time profile of oxazepam were studied applying the technique of direct cortical stimulation as a measure of the anticonvulsant effect (chapter 9). Using this technique, it was reported that no (inter)active metabolites are formed (Dingemanse et al., 1990). Since the effect vs. time profile in the younger animals suggested the occurrence of acute tolerance and/or withdrawal phenomena, a pilot study in young animals was performed to investigate whether the occurrence of tolerance and/or withdrawal really is a reliable explanation for the course of the effect vs. time profile. Next to the *in vivo* pharmacodynamics, the *in vitro* benzodiazepine receptor binding characteristics were investigated to study more mechanistically the increase in brain sensitivity found in the *in vivo* study.
In chapter 10 the pharmacodynamics of the anticonvulsant effect of sodium valproate were determined in rats of different age groups. The rationale for this study was the suggested impaired action of sodium valproate on the GABA system in the elderly (Monteleone et al., 1987). For most other drugs an increased action on the GABA system during ageing is found. In addition, the sensitivity to adverse effects of sodium valproate was reported to be higher in young subjects compared to older ones (Zafrani and Berthelot, 1982; Williams et al., 1984), whereas for most other drugs the sensitivity to adverse effects appears to be higher in the elderly. Because of this rationale, two extra age groups (young and middle-aged) were incorporated into this study to a total of seven groups. The plasma concentration vs. anticonvulsant effect profiles were determined during continuous intravenous infusion of sodium valproate. In order to gain insight into potential changes in distribution of the drug between plasma and the site of action the influence of ageing on the distribution of the drug between plasma (total and unbound

concentration), brain tissue and cerebrospinal fluid was determined at the end of the infusion. In a separate group of young animals the distribution of the drug between plasma (total and unbound) and cerebrospinal fluid was determined at several time points during infusion.

V Conclusions and perspectives

The results obtained in these investigations are briefly reviewed and some possibilities for further studies are discussed.

References

Bell, G.D., Spickett, G.P., Reeve, P.A., Morden, A. and Logan, R.F.A.: Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. Br. J. clin. Pharmacol. 23: 241-244, 1987.

Danhof, M. and Levy, G.: Kinetics of drug action in disease states. I. Effect of infusing rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 229: 44-50, 1984.

Dingemanse, J., Thomassen, D., Mentink B.H. and Danhof M.: Strategy to assess the role of (inter)active metabolites in pharmacodynamic studies in-vivo: a model study with heptabarbital. J. Pharm. Pharmacol. 40: 552-557, 1988.

Dingemanse, J., Hutson, P.H., Langemeijer, M.W.E., Curzon, G. and Danhof, M.: Application of serial sampling of cerebrospinal fluid in pharmacodynamic studies with a drug active in the CNS: Heptabarbital concentrations at onset and offset of loss of righting reflex in rats. Neuropharmacol. 27: 467-474, 1988a.

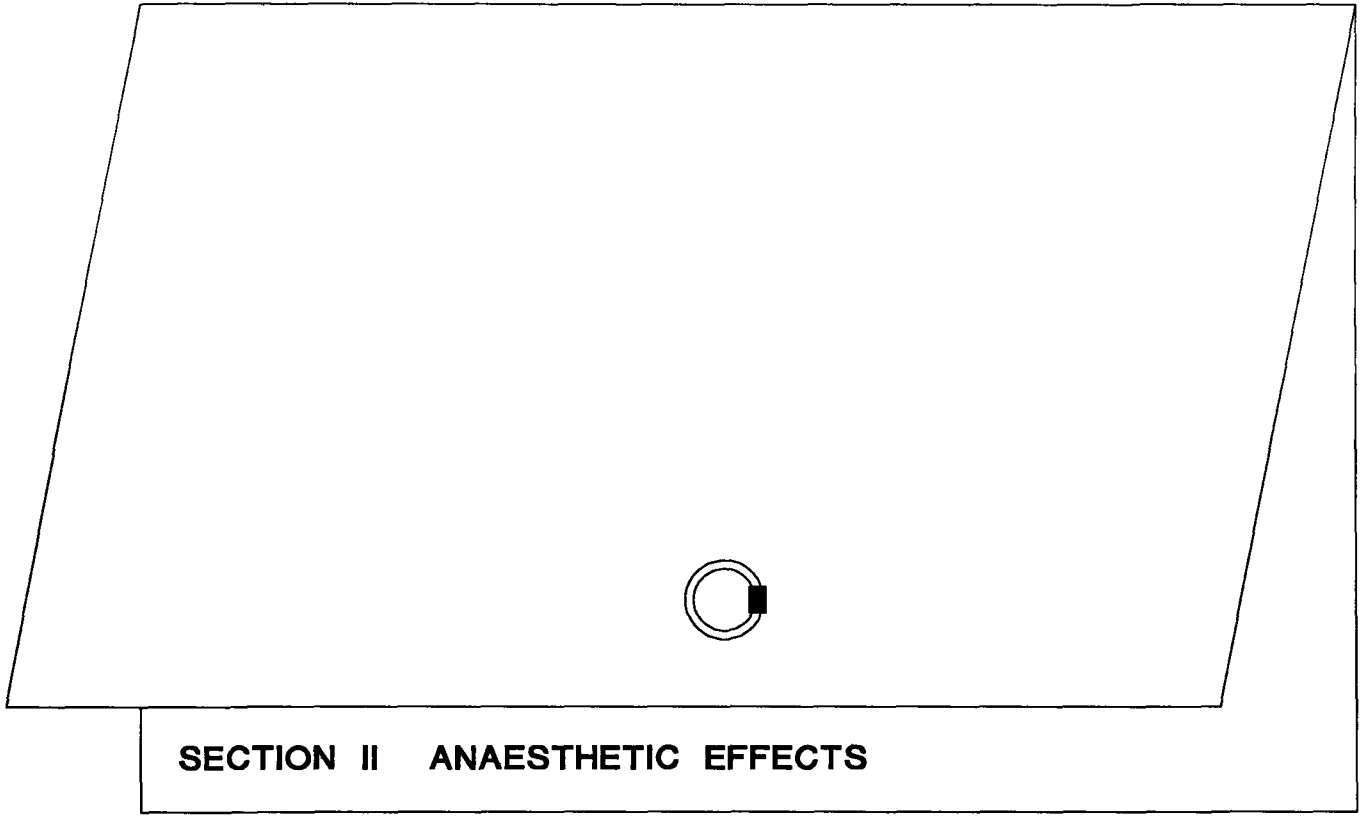
Dingemanse, J., Voskuyl, R.A., Langemeijer, M.W.E., Postel-Westra, I., Meinardi, H. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the anticonvulsant effect of oxazepam in individual rats. Br. J. Pharmacol. 99: 53-58, 1990.

Mandema, J.W., Tukker, E. and Danhof, M.: Pharmacokinetic/ pharmacodynamic modelling of EEG effects of midazolam in individual rats: influence of rate and route of administration. Br. J. Pharmacol. 102: 663-668, 1991.

Monteleone, P., Iovino, M., Orio, F. and Steardo, L.: Impaired growth hormone response to sodium valproate in normal ageing. Psychopharmacol. 91: 10-13, 1987.

Williams, C.A., Tiefenbach, S. and McReynolds, J.W.: Valproic acid-induced hyperammonemia in mentally retarded adults. Neurology 34: 550-553, 1984.

Zafrani, E.S. and Berthelot, P.: Sodium valproate in the induction of unusual hepatotoxicity. Hepatology 2: 648-649, 1982.



SECTION II ANAESTHETIC EFFECTS

Chapter 4

INCREASED SENSITIVITY TO THE ANAESTHETIC EFFECT OF PHENOBARBITAL IN AGEING BN/BIRIJ RATS

A.M. Stijnen[1,2], M. Danhof[1] and C.F.A. van Bezooijen[2]

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, The Netherlands
[2]TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands

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Summary

The purpose of this study was to determine the influence of age on the pharmacokinetic-pharmacodynamic relationship of phenobarbital in male BN/BiRij rats, aged 4, 15, 26, 31 and 36 months.
The pharmacokinetics were studied on basis of the plasma concentration vs. time profile and the excretion of phenobarbital and parahydroxyphenobarbital in urine and faeces following an intravenous dose of 20 mg/kg. The pharmacodynamics were determined as the threshold dose and cerebrospinal fluid threshold concentration of phenobarbital for the onset of loss of righting reflex (as a measure of the anaesthetic effect) during an intravenous infusion at a rate of 3 mg/min.
The dose requirement for the anaesthetic effect decreased with increasing age from 263 ± 4 mg/kg (mean ± SEM) for the 4-month-old rats to 202 ± 6 mg/kg for the 31-month-old rats. Only minor changes in the pharmacokinetic parameters, the metabolite profile and the distribution of phenobarbital

between plasma (total and free), cerebrospinal fluid and brain tissue were observed. With respect to the pharmacodynamics however, evidence for an increase in brain sensitivity was observed, as reflected in a decrease in the threshold cerebrospinal fluid concentration from 181 ± 4 mg/l (mean ± SEM) at 4 months to 134 ± 4 mg/l at 31 months.
It is concluded that the decreased dose requirement of phenobarbital in elderly BN/BiRij rats is due to an increased brain sensitivity, as a result of the ageing process per se rather than detectable pathology.

Introduction

It is well recognized that increasing age is associated with changes in the response to a wide variety of drugs acting on the central nervous system (Bender, 1979; Ouslander, 1981; Kaiko et al., 1982; Miller, 1987), which is also reflected in the high incidence of adverse drug reactions in the elderly. Methods of adverse drug reaction detection in controlled prospective drug monitoring and surveillance programs have identified drug dosage as a major determinant of adverse drug reactions in the elderly (Greenblatt et al.,1977). This suggests that with increasing age alterations in the dose vs. response relationship may occur. Significant changes in dose requirement have indeed been established for a number of drugs (Dundee, 1954; Belville et al., 1971; Christensen and Andreasen, 1978; Kaiko, 1980; Bell et al., 1987). In theory, changes in dose vs. response relationship can be accounted for by changes in pharmacokinetics, pharmacodynamics or a combination of both. Research has improved considerably our understanding of age-related changes in pharmacokinetics. Compared to the relative abundance of pharmacokinetic information in the elderly, information on age-related changes in pharmacodynamics is scarce. Studies on the effect of ageing on pharmacodynamics follow two approaches, one which applies the concepts of simultaneous pharmacokinetic-pharmacodynamic modelling to data obtained in humans (Homer and Stanski, 1985; Scott and Stanski, 1987) and one which involves *in vitro* pharmacodynamic studies on tissues obtained in animal models of ageing (Niles et al., 1988). Unfortunately, neither of these two approaches has thus far resulted in conclusive answers to the mechanisms and the magnitude of age-related changes in pharmacodynamics.
A problem with the studies conducted in man so far is that a number of

potentially confounding pharmacokinetic factors (i.e. presence of active metabolites, alterations in distribution and possible stereoselective changes in disposition) have not been taken *into consideration* (Dingemanse et al., 1988a,b). In addition, the relationship between the utilized effect measure in several studies (quantitative EEG parameters) and the true therapeutic actions and/or adverse drug reactions is still unknown. A problem with *in vitro* pharmacodynamic studies is that changes can occur simultaneously at different organization levels of the pharmacodynamic process and that it is difficult to translate changes observed at one of these levels quantitatively into changes at the pharmacodynamic level *in vivo* (Danhof, 1989).
In order to improve the understanding of age-related changes in pharmacodynamics, a combination of *in vivo* and *in vitro* studies is indicated. In this respect, the use of animal models of ageing appears to be indispensable.
The purpose of the present investigation was to examine the mechanism of the age-related change in dose requirement of barbiturates, which is well documented clinically (Dundee, 1954; *Christensen* et al., 1981, 1982, 1983). The study was conducted in BN/BiRij rats as an animal model of ageing. A realistic measure of the anaesthetic effect was used (loss of righting reflex) and an experimental strategy was used which allows a clear differentiation between age-related changes in pharmacokinetics on one hand and in pharmacodynamics on the other (Danhof and Levy, 1984).
Because it is known that renal dysfunction, which can occur in elderly animals, leads to an increased brain sensitivity for phenobarbital (Danhof et al., 1984a), kidney function is also considered in this study.

Materials and methods

Animals

Five groups of male BN/BiRij rats (TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands) of different ages (4, 15, 26, 31 and 36 months) were used. The 10, 50 and 90 % survival ages of this strain are 38.1, 31.7 and 22.8 months, respectively. During the period in which the experiments were performed, the rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature was maintained at 22-23 °C. They were allowed free

access to water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands), also during the experiments.

Chemicals

Phenobarbital sodium was purchased from Brocacef (Maarssen, The Netherlands), parahydroxyphenobarbital from Aldrich Chemie (Brussels, Belgium).

Animal experiments

1. Clinical biochemical/pathological evaluation

In order to be able to determine the health status of the animals, several clinical biochemical parameters were measured. Blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transpeptidase, glucose, triglycerides, cholesterol and haemoglobine were measured using a Reflotron Autoanalyser (Boehringer, Mannheim, W.Germany). Total protein concentrations in plasma were determined by means of the biuret reaction (Gornall et al., 1949), using bovine serum albumin as a standard. Albumin concentrations were determined by radial immunodiffusion, as described by Mancini et al. (1965) and modified by Radl et al. (1970), using rat albumin as a standard. Urine was collected during 24 hours while the rats were kept in metabolic cages. The volume of the urine was determined and the osmolality was measured using a Digimatic Osmometer 3D II (Advanced Instruments Inc., Mass., USA). The creatinine clearance was measured using the Sigma Test Kit No. 555-A (Sigma Chemical Co., St. Louis, MO, USA).

After the pharmacokinetic and pharmacodynamic evaluation, the animals were sacrificed, the liver was weighed and the heart, the lungs, the kidneys, the liver, the brain and macroscopically visible abnormalities were evaluated by a pathologist, who was unaware of the outcome of the pharmacokinetic and pharmacodynamic evaluation.

2. Pharmacokinetic evaluation

20 Mg/kg phenobarbital was administered i.v. via the penal vein under a light halothane anaesthesia. 11 Bloodsamples of 120 µl were taken from an incision in the tail in a period of 56 hours after administration. Urine and faeces were collected for 6 days in four fractions (day 1, day 2, day 3 and days 4, 5, 6) while the rats were kept in individual metabolic cages.

3. Pharmacodynamic evaluation

To minimize the influence of a possible diurnal rhythm in brain sensitivity and/or rate of metabolism (Roberts et al., 1970), the pharmacodynamic evaluations were performed between 9:00 A.M. and 1:00 P.M..

The evaluation was performed two weeks after the pharmacokinetic evaluation. One day before the experiment, the jugular vein was cannulated under halothane anaesthesia. Phenobarbital was infused via the jugular vein cannula at a rate of 3 mg/min until the point of loss of righting reflex (resulting in an infusion time of 18-32 min). At this point, cerebrospinal fluid, blood and brain tissue were collected. This evaluation was further performed as described by Danhof and Levy (1984).

Drug analysis

The concentrations of phenobarbital in plasma, cerebrospinal fluid and brain tissue were measured by an HPLC-method described by Danhof and Levy (1984). The concentrations of parahydroxyphenobarbital in urine and faeces were measured by an HPLC-method described by Dingemanse (1988). Protein binding of phenobarbital was determined by means of ultrafiltration using the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands). The concentrations of phenobarbital in the ultrafiltrate were measured in the same way as the plasma samples.

HPLC-apparatus

The HPLC-system, used for the determination of phenobarbital and parahydroxyphenobarbital consisted of either a Waters M6000 solvent delivery system (Waters Ass., Milford, U.S.A.) or a Kontron 420 pump (Zurich,

Switzerland), a WISP 710B automatic sample injector, and a M440 absorbance detector at 254 nm (both Waters Ass., Milford, U.S.A.) and a RCM-100 containing a Radial-Pak C-18 cartridge, particle size 10 µm (Waters Ass.). A Trivector CP 2000 chromatography data system (Chrompack, Middelburg, The Netherlands) was used to process the chromatographic data.

Data analysis

1. Pharmacokinetics

The area under the phenobarbital concentration-time curve (AUC) was calculated using the linear trapezoidal rule with extrapolation to infinity, using the elimination rate constant k. This elimination rate constant was determined using the slope of the terminal phase of the log concentration vs. time profile. The elimination half life was calculated as 0.693/k. Total body clearance was calculated as dose/AUC, renal clearance as percentage of the dose excreted unchanged in urine * total clearance, intrinsic total body clearance as total body clearance/fraction unbound in plasma, clearance of formation of parahydroxyphenobarbital as fraction of dose excreted as parahydroxyphenobarbital * total clearance and the apparent volume of distribution (V_{area}) as dose/AUC*k.

2. Statistics

The effect of ageing on the clinical biochemical, pharmacokinetic and pharmacodynamic parameters was statistically tested by one-way analysis of variance with the Student's t-test with Bonferroni correction used to examine differences between age groups. Bartlett's test was used to assess homogeneity of variances. In case of nonhomogeneity of variances the Welch test and the multiple Welch test were used. P-values lower than 0.05 were judged to be significant. The results of the 36-month-old animals were included in the statistical analysis of the results of the clinical biochemical/pathological and the pharmacokinetic evaluation (except for the intrinsic total body clearance), but not in that of the pharmacodynamic evaluation because of the low number of animals left (n=2).

In order to investigate the contribution of potential changes in pharmacokinetics, pharmacodynamics and kidney functioning to age-related changes in dose requirement, and the contribution of potential changes in

kidney functioning to age-related changes in pharmacodynamics, multiple regression was applied.

Results

1. Clinical biochemical/pathological evaluation

The results of the clinical biochemical indices are shown in table 1. In this table, the values for all animals that were included in the evaluation are given. Generally, very few changes were found in all parameters in both the mean and the individual values.

As indicators for the kidney function, the blood urea nitrogen concentration, the creatinine clearance, the osmolality of the urine and the urine production were measured. These last two parameters showed a tendency of a decreasing ability of the kidneys to reabsorb water from the ages of 15 months until 36 months. The low value of the creatinine clearance of the 36-month-old indicated a decreased glomerular filtration rate. The blood urea nitrogen concentration did not change during ageing. Considering all the data, the animals did not appear to suffer from impaired kidney functioning, since the values of the parameters for the kidney function were in the normal range for healthy animals (Van Bezooijen et al., 1974).

The pathological evaluation was performed in an attempt to differentiate between changes due to physiological ageing and changes as result of concomitant pathology. One 31-month-old animal appeared to suffer from a malign lymphoma in several organs, one 36-month-old animal suffered from nephropathy, a granular cell tumour in the brains and inflammations in several organs and another 36-month-old animal displayed signs of hyperplasia of the lungs, tubular and glomerular abnormalities of the kidneys and heart dysfunction. These three animals were excluded from the evaluations on the basis of these findings. Two of these three animals died (spontaneously) during the two week period in between the pharmacokinetic and the pharmacodynamic evaluation. In the pharmacokinetic evaluation, all three animals showed results in the normal range. However, the 36-month-old rat, suffering from nephropathy, a granular cell tumour and inflammations, appeared to be less sensitive to the anaesthetic effect of phenobarbital in the pharmacodynamic evaluation.

Table 1. Effect of age on selected clinical biochemical indices.

age (month)	4	15	26	31	36
number of animals	20	10	10	10	5
body weight (g)	243 ± 10	380 ± 6[a]	401 ± 6[a]	379 ± 3[a]	321 ± 15[a,c]
liver weight (g)	8.8 ± 0.4 (n=15)	11.1 ± 0.3[a]	11.9 ± 0.4[a]	12.1 ± 0.8[a]	11.8 ± 1.1 (n=4)
liver wt/ body wt (%)	3.14 ± 0.04 (n=15)	2.90 ± 0.04[a]	3.14 ± 0.07	3.42 ± 0.25	3.72 ± 0.17 (n=4)
blood aspartate aminotransferase (I.U./l)	89.9 ± 3.1	77.5 ± 2.9	74.3 ± 6.0	75.6 ± 5.6	92.2 ± 5.8
blood alanine aminotransferase (I.U./l)	39.1 ± 1.3	36.9 ± 1.0	32.2 ± 1.0[a,b]	38.7 ± 2.0	37.6 ± 3.7
plasma albumin (mg/ml)	34.8 ± 0.8	40.7 ± 2.1	43.5 ± 2.6	31.3 ± 1.0[b,c]	42.6 ± 2.3
plasma total protein (mg/ml)	89.0 ± 1.2	95.3 ± 1.5[a]	97.0 ± 1.1[a]	92.8 ± 1.8	95.7 ± 2.5
blood glucose (mmol/l)	5.5 ± 0.2	5.7 ± 0.3	4.6 ± 0.4	5.3 ± 0.4	4.3 ± 0.3
hemoglobine (mmol/l)	8.6 ± 0.4	8.5 ± 0.2	8.2 ± 0.3	8.4 ± 0.4	7.5 ± 0.4
blood γ-glutamyltranspeptidase (I.U./l)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
blood cholesterol (mmol/l)	< 2.7	< 2.7	2.8 ± 0.1	< 2.7	< 2.8
blood triglycerides (mmol/l)	< 0.9	< 1.0	1.2 ± 0.1	1.3 ± 0.2	< 1.0
blood urea nitrogen (mmol/l)	7.7 ± 0.2	7.4 ± 0.1	7.2 ± 0.2	8.0 ± 0.4	9.4 ± 1.7
creatinine clearance (ml/h.kg)	278 ± 21 (n=17)	232 ± 24	230 ± 26	189 ± 18	144 ± 13[a]
urine production (ml/24 hrs)	9.0 ± 0.5	6.0 ± 0.4[a]	7.8 ± 0.5	11.5 ± 1.5	15.3 ± 3.4
osmolality (mOsm/l)	1705 ± 65	1739 ± 81	1340 ± 76[a,b]	1069 ± 79[a,b]	870 ± 150[a,b]

Results are presented as mean ± SEM

[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 15-month value, $p < 0.05$
[c] significantly different from 26-month value, $p < 0.05$

2. Pharmacokinetic evaluation

The values of several pharmacokinetic parameters in the different age groups are summarized in table 2.

As a whole, only minor age-related changes in the pharmacokinetics were observed. A slight decrease in both total and intrinsic clearance of phenobarbital was observed with increasing age. Similar findings were

Table 2. *The influence of ageing on the pharmacokinetic parameters of phenobarbital in rats following an intravenous bolus dose of 20 mg/kg.*

age (month)	4	15	26	31	36
number of animals	20	10	10	9	3
total body clearance (ml/h.kg)	55.8 ± 2.0	46.3 ± 2.2	44.7 ± 2.6[a]	38.3 ± 2.3[a]	39.7 ± 6.4
intrinsic total body clearance (ml/h.kg)	68.0 ± 2.2	57.0 ± 2.3[a]	57.1 ± 3.5 (n=9)	48.1 ± 3.3[a] (n=7)	58.4 / 30.7[A]
renal clearance (ml/h.kg)	7.8 ± 0.6 (n=13)	4.9 ± 0.3[a]	2.9 ± 0.4[a,b] (n=9)	3.1 ± 0.4[a,b]	3.7 ± 0.6
clearance of formation of parahydroxyphenobarbital (ml/h.kg)	31.0 ± 1.6 (n=14)	26.1 ± 1.7	23.9 ± 2.6 (n=9)	19.5 ± 1.6[a]	21.6 ± 3.8
volume of distribution (ml/kg)	693 ± 34	662 ± 35	625 ± 39	586 ± 17	770 ± 160
elimination half-life (h)	8.6 ± 0.2	10.1 ± 0.7	9.8 ± 0.6	11.1 ± 1.0	13.5 ± 1.6

The results are presented as mean ± SEM

[A] individual values (for the intrinsic total body clearance the statistical analysis was performed without using the values of the 36-month-old rats)
[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 15-month value, $p < 0.05$

obtained for the metabolic clearance (clearance for formation of parahydroxyphenobarbital). Renal clearance was found to decrease significantly from 7.8 ± 0.6 to 2.9 ± 0.4 ml/h.kg (mean ± SEM) in the age groups of 4 and 26 months, respectively, and seemed to be relatively constant thereafter. The elimination half-life showed a tendency to increase with age.

The pattern of metabolism of phenobarbital was studied on the basis of the profile of excretion of phenobarbital and parahydroxyphenobarbital in urine and faeces (figure 1). Parahydroxyphenobarbital was found to be the major metabolite accounting for approximately 55 % of the administered dose excreted in urine and faeces. The total amount of phenobarbital and parahydroxyphenobarbital excreted amounted to 70 % of the administered dose. With increasing age only minor changes in the excretion profile were observed.

The volume of distribution was 693 ± 34 ml/kg in the 4-month-old animals and did not change with increasing age (table 2). In addition, only minor changes in the distribution of phenobarbital between plasma, brain tissue and cerebrospinal fluid were observed (table 3).

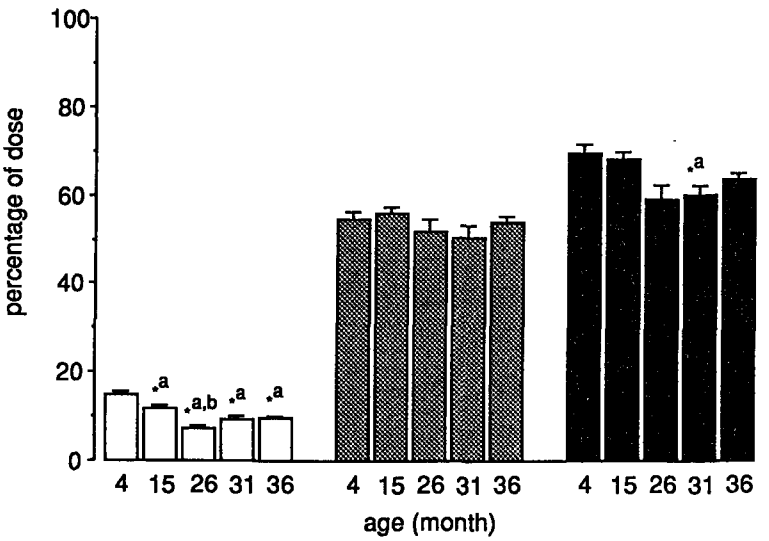

Figure 1. The influence of ageing on the excretion of phenobarbital and parahydroxyphenobarbital in urine and faeces following an intravenous dose of 20 mg/kg.

□ percentage of dose recovered in urine and faeces unchanged

▨ percentage of dose recovered in urine and faeces as parahydroxyphenobarbital

■ total percentage of dose recovered in urine and faeces unchanged and as parahydroxyphenobarbital

all values are expressed as mean ± SEM; number of animals was 14, 10, 9, 9 and 3 for the 4-, 15-, 26-, 31-, and 36-month-old group, respectively

*a significantly different from 4-month value, $p < 0.05$
*b significantly different from 15-month value, $p < 0.05$

3. Pharmacodynamic evaluation

The values for the threshold dose of phenobarbital required to reach loss of righting reflex and the threshold concentrations in different compartments at that point are given in table 4. One of the 36-month-old animals died before the pharmacodynamic evaluation was performed. Therefore, the two 36-month-old animals were not included in the statistical evaluation.

Table 3. *The influence of ageing on the relative distribution of phenobarbital at onset of loss of righting reflex.*

age (month)	4	15	26	31	36
number of animals	20	10	9	7	2
conc. csf/conc. plasma free[B]	0.69 ± 0.02 (n=18)	0.72 ± 0.02	0.66 ± 0.03	0.60 ± 0.02[b] (n=6)	0.56 / 0.77[A]
conc. csf/conc. plasma total	0.57 ± 0.01 (n=18)	0.59 ± 0.02	0.52 ± 0.03	0.47 ± 0.01[a,b] (n=6)	0.45 / 0.67
conc. csf/conc. brain	0.65 ± 0.01 (n=18)	0.67 ± 0.03	0.62 ± 0.02	0.62 ± 0.01 (n=6)	0.60 / 0.74
plasma protein binding (%)	18.8 ± 1.6 (n=19)	18.7 ± 2.2	20.5 ± 1.7	21.6 ± 1.8	20.2 / 12.1
conc. brain/conc. plasma free	1.03 ± 0.02	1.08 ± 0.02	1.05 ± 0.03	0.98 ± 0.03[b]	0.94 / 1.04
conc. brain/conc. plasma total	0.87 ± 0.01	0.89 ± 0.02	0.83 ± 0.02	0.77 ± 0.01[a,b]	0.75 / 0.92

The results are presented as mean ± SEM

[A] individual values; n=2 (the statistical analysis was performed without using the values of the 36-month-old rats)
[B] conc. = concentration; csf = cerebrospinal fluid

[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 15-month value, $p < 0.05$

Table 4. *The influence of ageing on dose and concentrations of phenobarbital at onset of loss of righting reflex during an intravenous infusion at a rate of 3 mg/min.*

age (month)	4	15	26	31	36
number of animals	20	10	9	7	2
threshold dose (mg/kg)	263 ± 4	224 ± 6[a]	205 ± 4[a]	202 ± 6[a]	181 / 175[A]
plasma concentration (mg/l)	327 ± 9	291 ± 9	277 ± 10[a]	284 ± 9	250 / 204
plasma ultrafiltrate concentration (mg/l)	267 ± 4	236 ± 5[a]	220 ± 6[a]	222 ± 8[a]	199 / 179
cerebrospinal fluid concentration (mg/l)	181 ± 4 (n=18)	170 ± 5	143 ± 4[a,b]	134 ± 4[a,b] (n=6)	112 / 138
brain concentration (mg/kg)	283 ± 5	255 ± 8[a]	230 ± 7[a]	218 ± 8[a,b]	188 / 187

The results are presented as mean ± SEM

[A] individual values; n=2 (the statistical analysis was performed without using the values of the 36-month-old rats)

[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 15-month value, $p < 0.05$

The threshold dose for induction of loss of righting reflex decreased between 4 and 31 months. The concentration of phenobarbital at onset of loss of righting reflex in cerebrospinal fluid and in brain tissue also decreased from the age of 4 months until the age of 31 months. The concentration in plasma (total and free) only decreased until the age of 26 months. Multiple regression showed that the decrease of the phenobarbital threshold dose and threshold concentrations with increasing age was not due to impaired kidney functioning.

Figure 2 shows the relationship between cerebrospinal fluid threshold concentration and the threshold dose. The correlation coefficient for linear regression was 0.83 ($p < 0.001$). Multiple regression showed that the factors age and phenobarbital cerebrospinal fluid concentration contribute significantly to the decrease in threshold dose, whereas the volume of distribution, the total body clearance and the parameters for kidney functioning did not.

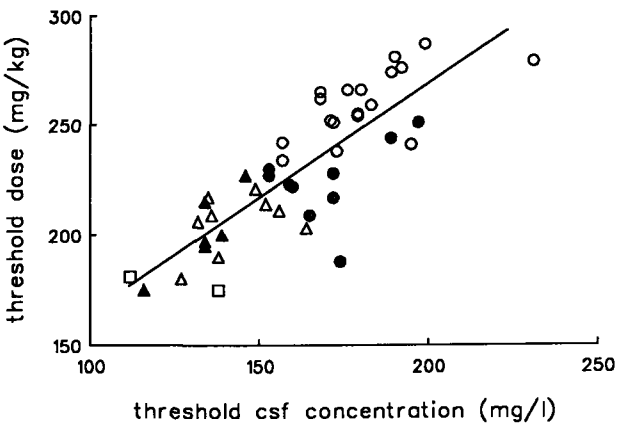

Figure 2. Contribution of pharmacokinetic and pharmacodynamic changes to change in dose requirement with ageing.
Threshold dose vs. concentration in cerebrospinal fluid at onset of loss of righting reflex, correlation coefficient = 0.83 ($p < 0.001$)

The values of all individual animals are plotted, for the five different ages different symbols are used: ○ 4-month-old, ● 15-month-old, △ 26-month-old, ▲ 31-month-old, □ 36-month-old

Discussion

In the present study, the influence of ageing on the pharmacodynamics of phenobarbital was investigated, using an experimental strategy, which enables a clear distinction between changes in pharmacokinetics and pharmacodynamics. The point of loss of righting reflex was used as a measure for the anaesthetic effect. The threshold dose needed to attain loss of righting reflex appeared to decrease steadily during ageing. This finding is in agreement with the human situation for thiopental (Dundee, 1954; Christensen et al., 1981, 1982, 1983; Muravchick, 1984; Homer and Stanski, 1985; Stanski and Maitre, 1990).
An important question is whether this change in dose requirement is due to a change in pharmacokinetics, pharmacodynamics or a combination of both. For the human situation for thiopental, conflicting data with regard to the influence of ageing on the pharmacodynamics have been reported (Oduah, 1969; Christensen et al., 1981, 1982, 1983; Homer and Stanski, 1985; Stanski and Maitre, 1990). In these studies however, one or more pharmacokinetically complicating factors have not been taken into account, such as protein binding (Jung et al., 1982), active metabolites (Dundee, 1974) and stereoselective disposition of the enantiomers (Soudijn, 1983).

In the design used in this study in order to measure the dose requirement, the volume of distribution is the most important pharmacokinetic parameter. Phenobarbital was infused at a rate of 3 mg/min until onset of loss of righting reflex. This resulted in an infusion time of 18-32 min. Because the elimination half-life varied from 8.6 hours for the young animals to 13.5 hours for the oldest animals, the elimination process was too slow to influence the threshold dose measured within 32 minutes after the start of the administration. While the volume of distribution was not changing during ageing, the decrease in the threshold dose appeared not to be due to a change in the pharmacokinetics.

The results of the pharmacodynamic evaluation showed a decrease in the concentration in cerebrospinal fluid, in brain tissue and in plasma (total and free) at onset of loss of righting reflex. These findings are suggestive for an increase in brain sensitivity with increasing age.
However, as already mentioned in the introduction, it is important to account

for pharmacokinetically confounding factors in pharmacodynamic studies, being age-related changes in protein binding, in distribution and in formation of (inter)active metabolites. In the present study, the protein binding was constant for the several age groups. The distribution of phenobarbital between cerebrospinal fluid, brain tissue and plasma did not display marked differences. The tendency towards decreased ratios in the 31-month-old animals might be explained by a decrease in cerebral blood flow (Meyer, 1986, Takeda et al., 1988).

The ratio cerebrospinal fluid/plasma free being < 1 implied a situation of non-equilibrium at onset of loss of righting reflex (18-32 minutes after drug administration) and the importance of measuring cerebrospinal fluid concentrations.

As the concentration ratios decreased rather than increased with increasing age, it seems that a change in the distribution between blood and the central nervous system cannot explain the decreased dose requirement in the elderly animals. Also it is important that the pharmacodynamic evaluation was based upon cerebrospinal fluid, which means that concentrations that are in rapid equilibrium with the site of action have been measured.

The third confounding factor in studying pharmacodynamics is the formation of (inter)active metabolites. In our study, the metabolism and excretion of phenobarbital were studied in the different age groups by measuring the amount of phenobarbital excreted unchanged in urine and in faeces and the amount of parahydroxyphenobarbital excreted in urine and in faeces.

The purpose of these measurements was to determine whether large qualitative changes in metabolism occur. In previous studies, it was demonstrated in young animals that parahydroxyphenobarbital is not an active or interactive metabolite (Danhof and Levy, 1984). In the present study, the total recovery of phenobarbital and parahydroxyphenobarbital did not change with age, which indicates that the metabolism did not change qualitatively and no new active metabolites were formed. This means that the conclusion by Danhof and Levy (1984) about the anaesthetic effect being exerted only by phenobarbital itself, also holds for the elderly animals. Also the pharmacodynamics were determined during infusion and the duration of the infusion was relatively short compared to the elimination half-life. Therefore, metabolite formation was of virtually no importance in the determination of the threshold dose and concentration.

The three potentially pharmacokinetically confounding factors in our

pharmacodynamic study, protein binding, distribution and formation of (inter)active metabolites, appeared not to show age-related changes. This implies that from the decreasing concentration of phenobarbital in cerebrospinal fluid at onset of loss of righting reflex with increasing age can be concluded that the sensitivity of the brain for the anaesthetic effect of phenobarbital increases with age.

Danhof et al. (1984a) reported a change in the pharmacodynamics of phenobarbital in animals with experimental renal dysfunction. Experimental liver disease and experimental diabetes did not have an influence on the pharmacodynamics of this compound (Danhof et al., 1985, 1985a). The BN/BiRij rats are known to develop an age-related impairment of the kidney function, whereas the liver is hardly affected by the ageing process (Burek, 1978). It is important that the magnitude of the change in brain sensitivity is similar in the study of Danhof et al. and the present study, but that there were major differences in renal functioning. The values of the parameters for the kidney function measured in our study were in the normal range for healthy animals (Van Bezooijen et al., 1974). Multiple regression showed that the increased sensitivity for phenobarbital with increasing age is not caused by kidney dysfunctioning.

Wanwimolruk and Levy (1987) investigated the influence of ageing on the pharmacodynamics of phenobarbital in male Sprague-Dawley rats (aged 4-5 weeks, 9 months and 18 months, n=6 for each group) and in a small number of Fischer-344 rats (aged 7, 16 and 24 months, n = 3, 3 and 2, respectively). They also measured the anaesthetic effect using the technique of loss of righting reflex and observed a decrease in threshold dose and cerebrospinal fluid threshold concentration with increasing age Wanwimolruk and Levy, 1987). A direct comparison between those studies is difficult, because no data were reported on the survival curves in both strains of rats and no post-mortem tissue examination was performed (only a few clinical biochemical parameters were measured). In a study we performed with heptabarbital (Stijnen et al., submitted; chapter 6), we found important pathological abnormalities already in 12- and 24-month-old animals and observed that rats with pathological abnormalities, that did show different pharmacokinetic and pharmacodynamic behaviour compared to the healthy ones, did not always display abnormal values for the biochemical parameters, so it is important not

only to measure those parameters but also to perform a post-mortem tissue examination.
Unfortunately, Wanwimolruk and Levy only measured until the age of 24 months. It is very important to include very old animals, because it is quite possible that the major age-related changes only appear at a high age (Bell et al., 1987).

It is imaginable that the increase in brain sensitivity for the anaesthetic effect of phenobarbital can be (partly) explained by a non-specific effect on the cerebral oxygen consumption. The decrease in cerebral oxygen consumption produced by barbiturates correlates closely with the degree of anaesthesia (Michenfelder, 1974). Baughman et al. (1986) reported that a dose of 250 mg/kg phenobarbital produced a higher decrease of the cerebral oxygen consumption in 28-month-old Wistar rats compared to 6-month-old rats, suggesting that high-dose phenobarbital may depress nonelectrical cerebral metabolic processes more in aged rats.

In this study we have found that the threshold dose for the anaesthetic effect of phenobarbital, measured using the technique of loss of righting reflex, decreased during ageing, as is the case for thiopental in humans. The results of these investigations can be unambiguously interpreted as an increase in the brain sensitivity to the anaesthetic effect of phenobarbital, which is apparently not related to detectable pathology. This increase in brain sensitivity is the predominant cause of the decrease in the anaesthetic threshold dose of phenobarbital with increasing age measured using the technique of loss of righting reflex.

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References

Baughman, V.L., Hoffman, W.E., Miletich, D.J. and Albrecht, R.F.: Effects of phenobarbital on cerebral blood flow and metabolism in young and aged rats. Anesthesiology 65: 500-505, 1986.

Becker, K.E.: Plasma levels of thiopental necessary for anesthesia. Anesthesiology 49: 192-196, 1978.

Bell, G.D., Spickett, G.P., Reeve, P.A., Morden A. and Logan, R.F.A.: Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. Br. J. clin. Pharmacol. 23: 241-244, 1987.

Bellville, J.W., Forrest, W.H., Miller, E. and Brown, B.W.: Influence of age on pain relief from analgetics: A study of postoperative patients. J. Am. Med. Assoc. 217: 1835-1841, 1971.

Bender, A.D.: Drug sensitivity in the elderly. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson, eds., The Macmillan Press Ltd., 1979, pp 147-153.

Burek, J.D.: Pathology of aging rats. A morphological and experimental study of the age-associated lesions in aging BN/Bi, Wag/Rij and (WAG x BN) F_1 rats. CRC-Press Inc., West Palm Beach, Florida, 1978.

Christensen, J.H. and Andreasen, F.: Individual variation in response to thiopental. Acta Anaesthesiol. Scand. 22: 303-313, 1978.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Influence of age and sex on the pharmacokinetics of thiopentone. Br. J. Anaesth. 53: 1189-1195, 1981.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Pharmacokinetics and pharmacodynamics of thiopentone. Anaesthesia 37: 398-404, 1982.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Thiopentone sensitivity in young and elderly women. Br. J. Anaesth. 55: 33-40, 1983.

Danhof, M. and Levy, G.: Kinetics of drug action in disease states. I. Effect of infusing rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 229: 44-50, 1984.

Danhof, M., Hisoaka, M. and Levy G.: Kinetics of drug action in disease states. II. Effect of experimental renal dysfunction on phenobarbital concentrations in rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 230: 627-631, 1984a.

Danhof, M., Hisoaka, M. and Levy, G.: Kinetics of drug action in disease states. VI Effect of experimental diabetes on phenobarbital concentrations in rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 232: 435-438, 1985.

Danhof, M., Hisoaka, M. and Levy, G.: Kinetics of drug action in disease states. XII Effect of experimental liver diseases on the pharmacodynamics of phenobarbital and ethanol in rats. J. Pharm. Sci. 74: 321-324, 1985a.

Danhof, M.: Does variability in drug disposition explain (all) variability in drug effects? in: Topics in Pharmaceutical Sciences 1989. D.D. Breimer, K.K. Midha, D.J.A. Crommelin, eds., Amsterdam Medical Press, Noordwijk, The Netherlands, 1989, pp 573-586.

Dingemanse, J.: Pharmacokinetic-pharmacodynamic modelling of drug effects on the central nervous system. Thesis, University of Leiden, J.H. Pasmans B.V., The Hague, The Netherlands, 1988, pp189-202.

Dingemanse, J., Thomassen, D., Mentink B.H. and Danhof M.: Strategy to assess the role of (inter)active metabolites in pharmacodynamic studies in-vivo: a model study with heptabarbital. J. Pharm. Pharmacol. 40: 552-557, 1988a.

Dingemanse, J., Hutson, P.H., Langemeijer, M.W.E., Curzon, G. and Danhof, M.: Application of serial sampling of cerebrospinal fluid in pharmacodynamic studies with a drug active in the CNS: Heptabarbital concentrations at onset and offset of loss of righting reflex in rats. Neuropharmacol. 27: 467-474, 1988b.

Dundee, J.W.: The influence of body weight, sex and age on the dosage of thiopentone. Br. J. Anaesth. 26: 164-173, 1954.

Dundee, J.W.: Biotransformation of thiopental and other thiobarbiturates. Int. Anaesthesiol. Clin. 12: 121-133, 1974.

Gornall, A.C., Bardawill, C.J. and David, M.M.: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 11: 751-766, 1949.

Greenblatt, D.J., Allen, M.D. and Shader, R.I.: Toxicity of high-dose flurazepam in the elderly. Clin. Pharmacol. Ther. 21: 355-361, 1977.

Homer, T.D. and Stanski, D.R.: The effect of increasing age on thiopental disposition and anesthetic requirement. Anesthesiology 57: 714-724, 1985.

Jung, D., Mayersohn, M., Perrier, D., Calkins, J. and Saunders, R.: Thiopental disposition as a function of age in female patients undergoing surgery. Anesthesiology 57: 1079-1089, 1982.

Kaiko, R.F.: Age and morphine analgesia in cancer patients with postoperative pain. Clin. Pharmacol. Ther. 28: 823-826, 1980.

Kaiko, R.F., Wallenstein, S.L., Rogers, A.G., Grabinski, P.Y., Houde, R.W.: Narcotics in the elderly. Med. Clin. North America 66: 1079-1089, 1982.

Mancini, G., Carbonara, A.O. and Heremans, J.F.: Immunochemical quantitation of antigens by single radial immunodiffusion. Immunochemistry 2: 235, 1965.

Meyer, J.S.: Recent advances in measuring cerebral blood flow and metabolism in human aging, cerebrovascular disease, dementia, sleep and the epilepsies. in: Cerebrovascular Disease: Research and Clinical Management, vol 1. H. Lechner, J.S. Meyer, E. Ott, eds., Elsevier Science Publishers B.V. (Biomedical Division), 1986, pp 67-86.

Michenfelder, J.D.: The interdependency of cerebral function and metabolic effects following massive doses of thiopental in the dog. Anesthesiology 41: 231-236, 1974.

Miller R.: Anesthesia for the aged: Some pharmacokinetic and pharmacodynamic considerations. The Mount Sinai J. Med. 54: 305-307, 1987.

Muravchick, S.: Effect of age and premedication on thiopental sleep dose. Anesthesiology 61: 333-336, 1984.

Niles, L.P., Pulido, O.M. and Pickering, D.S.: Age-related changes in GABA and benzodiazepine receptor binding in rat brain are influenced by sampling time. Prog. Neuro-Psychopharmacol. Biol. Psychiat. 12: 337-344, 1988.

Oduah, M.: Effektivität und Wirkungsdauer von Thiopental beim Menschen in Abhängigkeit vom Alter. Der Anaesthesist 18: 308-310, 1969.

Ouslander, J.G.: Drug therapy in the elderly. Ann. of Int. Med., 95: 711-722, 1981.

Radl, J., Skvaril, F., Masopust, J. and Kithier, K.: Quantitative estimation of human immunoglobulins. II. The development of serum immunoglobulin levels in man. J. Hyg. Micribiol. Immun. 14: 488, 1970.

Roberts, P., Turnbull, M.J. and Winterburn, A.: Diurnal variation in sensitivity to and metabolism of barbiturate in the rat: lack of correlation between *in vivo* and *in vitro* findings. Eur. J. Pharmacol. 12: 375-377, 1970.

Scott, J.C. and Stanski, D.R.: Decreased fentanyl and alfentanil dose requirements with age. A simultaneous pharmacokinetic and pharmacodynamic evaluation. J. Pharmacol. Exp. Ther. 240: 159-166, 1987.

Shanks, C.: General anesthesia. in: Clinical pharmacology in the elderly. C.G. Swift, ed., Marcel Dekker, Inc., New York and Basel, 1987, pp 511-527.

Soudijn, W.: Advantages and disadvantages in the application of bioactive racemates or specific isomers as drugs. in: Stereochemistry and biological activity of drugs. E.J. Ariëns, W. Soudijn and P.B.M.W.M. Timmermans, eds., London, Blackpool, 1983, pp 89-102.

Stanski, D.R. and Maitre P.O.: Population pharmacokinetics and pharmacodynamics of thiopental: The effect of age revisited. Anesthesiology 72: 412-422, 1990.

Stijnen, A.M., Velders-van de Voort, S.H., Zurcher, C., Danhof, M. and Van Bezooijen, C.F.A.: The influence of concurrent disease on age-related changes in the pharmacokinetic-

pharmacodynamic relationship of heptabarbital in rats. submitted for publication.

Swift, C.G. and Triggs, E.J.: Clinical pharmacokinetics in the elderly. in: Clinical pharmacology in the elderly. C.G. Swift, ed., Marcel Dekker, Inc., New York and Basel, 1987, pp 31-82.

Takeda, S., Matsuzawa, T. and Matsui, H.: Age-related changes in regional cerebral blood flow and brain volume in healthy subjects. J. Am. Geriatr. Soc. 36: 293-297, 1988.

Van Bezooijen, K.F.A., De Leeuw-Israel, F.R. and Hollander, C.F.: Long-term functional aspects of syngeneic, orthotopic rat kidney grafts of different ages. J. Geront. 29: 11-19, 1974.

Wanwimolruk, S. and Levy, G.: Effect of age on the pharmacodynamics of phenobarbital and ethanol in rats. J. Pharm. Sci. 76: 503-507, 1987.

Chapter 5

AGE AND THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF PHENOBARBITAL IN RATS: 'PSEUDO'-LONGITUDINAL VS. CROSS-SECTIONAL STUDY DESIGN

A.M. Stijnen[1,2], M. Danhof[1] and C.F.A. van Bezooijen[2]

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, Leiden, The Netherlands
[2]TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands

submitted for publication

Summary

In a previous study, an apparent increase in brain sensitivity to the anaesthetic effect of phenobarbital was observed in an animal model of ageing. However, since this study was conducted according to a cross-sectional design, the observed change could, in principle, also have been the result of a cohort effect. The purpose of the present investigation was to exclude the role of such a cohort effect by adopting a 'pseudo'-longitudinal study design.

In this design one group of 45 animals, born within a period of two weeks, was reserved for the study and at five different ages (7, 14, 21, 29 and 34 months) one subgroup was investigated. A total of 37 animals was used; 8 animals died before the subgroup, to which they had been assigned at the start of the experiment, would be studied. A decrease in the anaesthetic threshold dose of phenobarbital was found during ageing, which appeared

mainly to be due to a decrease in the threshold concentration at the site of action. This decrease in the threshold concentration reflected an age-related increase in the sensitivity of the brain to the anaesthetic effect of phenobarbital.
It is concluded that the previously observed increase in brain sensitivity is indeed the result of the ageing process rather than a cohort effect in the animal model of ageing.

Introduction

Increasing age appears to be associated with changes in the sensitivity to a large number of drugs, which is reflected in alterations in dose requirement (Bender, 1979; Kaiko et al., 1982; Christensen et al., 1983; Miller, 1987; Stanski and Maitre, 1990). In order to further investigate the mechanism of these changes in sensitivity in terms of pharmacokinetics and pharmacodynamics, we recently conducted a series of studies with drugs acting on the central nervous system in an animal model of ageing. In one of these studies, an apparent increase in brain sensitivity to the anaesthetic effect of phenobarbital as measured on the basis of the 'loss of righting reflex technique' was observed (Stijnen et al., in press; chapter 4). This investigation, however, was conducted according to a cross-sectional design (different groups of rats aged between 4 and 36 months), which means that the observed change could in principle also have been the result of a 'cohort' effect. Considerable differences have been observed in physiology between cohorts as is reflected in for example the maximal survival (Schlettwein-Gsell, 1970; Curcio et al., 1984). For male BN/BiRij rats, which were also used in our studies, Mos and Hollander (1987) found extensive variation in both the 50% survival (ranging from 23.5 to 33.2 months) and the maximal survival (ranging from 34.6 to 39.9 months) between cohorts, but neither a systematic trend over the years and nor a consistent effect of the season of birth. Differences between the outcome of cross-sectional and longitudinal study designs have been reported for the body composition in young and old Sprague-Dawley rats (Lesser et al., 1973).
The purpose of the present investigations was to exclude the role of such cohort effects by adopting a 'pseudo'-longitudinal study design in a study on the brain sensitivity to the anaesthetic effect of phenobarbital. In this design one group of animals, born within a period of two weeks, was reserved for the

study and at five different ages one subgroup was investigated.

Materials and methods

Animals

45 male BN/BiRij rats (TNO-IVVO, Leiden, The Netherlands), born in the 45th, 46th or 48th week of 1988 were used. At the time of the start of the study, they were assigned to five different groups to be studied at the ages of 7, 14, 21, 28 or 34 months, respectively. Five groups of eight 5- to 7-month-old animals served as controls to detect possible differences caused by the experimental conditions in the time of the year during which the experiments were performed or potential cohort differences. The 10, 50 and 90 % survival ages of the BN/BiRij rats are 38.1, 31.7 and 22.8 months, respectively. Until the time of the experiment, they were kept in groups of two animals or solitary and during the period in which the experiments were performed, the rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature of the room was maintained at 22-23 °C. They were allowed free access to water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands), also during the experiments.

Chemicals

Phenobarbital sodium was purchased from Brocacef (Maarssen, The Netherlands).

Animal experiments

1. Clinical biochemical/pathological evaluation

In order to be able to determine the health status of the animals, several clinical biochemical parameters were measured. Blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase and glucose were measured using a Reflotron Autoanalyser (Boehringer, Mannheim,

W.Germany). Total protein concentrations in plasma were determined by means of the biuret reaction (Gornall et al., 1949), using bovine serum albumin as a standard. Albumin concentrations were measured spectrofotometrically using 2-(4'-hydroxybenzeneazo) benzoic acid (Ness et al., 1965 and Martinek, 1965). Rat albumin was used as a standard. Urine was collected during 24 hours while the rats were kept in metabolic cages. The volume of the urine was determined and the osmolality was measured using a Digimatic Osmometer 3D II (Advanced Instruments Inc., Mass., USA). The creatinine clearance was measured using the Sigma Test Kit No. 555-A (Sigma Chemical Co., St. Louis, MO, USA).
After the pharmacokinetic and pharmacodynamic evaluation, the animals were sacrificed; the liver was weighed; and the heart, the lungs, the kidneys, the liver, the brain and macroscopically visible abnormalities were evaluated by a pathologist, who was unaware of the outcome of the pharmacokinetic and pharmacodynamic evaluation.

2. Pharmacokinetic evaluation
Phenobarbital was administered as an intravenous bolus dose of 20 mg/kg via the penal vein under a light halothane anaesthesia. From an incision in the tail, 11 blood samples of 120 µl were taken in a period of 48 hours after administration.

3. Pharmacodynamic evaluation
The evaluation was performed two weeks after the pharmacokinetic evaluation. One day before the experiment, the jugular vein was cannulated under halothane anaesthesia. Phenobarbital was infused via the jugular vein cannula at a rate of 3 mg/min until the point of loss of righting reflex. At this point, cerebrospinal fluid, blood and brain tissue were collected (Danhof and Levy, 1984).
To minimize the influence of a possible diurnal rhythm in brain sensitivity and/ or metabolism rate (Roberts et al., 1970), the pharmacodynamic evaluation was performed between 9:00 A.M. and 1:00 P.M..

Drug analysis

The concentrations of phenobarbital in plasma, cerebrospinal fluid and brain tissue were measured by an HPLC-method described by Danhof and Levy (1984). Protein binding of phenobarbital was determined by means of ultrafiltration and the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands). The concentrations of phenobarbital in the ultrafiltrate were measured in the same way as that for the plasma samples.

HPLC-apparatus

The HPLC-system, used for the determination of phenobarbital and parahydroxyphenobarbital consisted of either a Waters M6000 solvent delivery system (Waters Ass., Milford, U.S.A.), a WISP 710B automatic sample injector, and a M440 absorbance detector at 254 nm (both Waters Ass., Milford, U.S.A.) and a RCM-100 containing a Radial-Pak C-18 cartridge, particle size 10 µm (Waters Ass.). A Trivector CP 2000 chromatography data system (Chrompack, Middelburg, The Netherlands) was used to process the chromatographic data.

Data analysis

1. Pharmacokinetics

The area under the phenobarbital concentration-time curve (AUC) was calculated using the linear trapezoidal rule extrapolated to infinity, on basis of the elimination rate constant k. This elimination rate constant was determined using the slope of the terminal phase of the log concentration vs. time profile. The elimination half life was calculated as 0.693/k. Total body clearance was calculated as dose/AUC and the apparent volume of distribution as dose/AUC*k.

2. Statistics

The effect of ageing on the pharmacokinetic and the pharmacodynamic parameters was statistically tested by one-way analysis of variance with the student-t test with Bonferroni correction used to examine differences between age groups. Bartlett's test was used to assess homogeneity of variances. In

case of nonhomogeneity of variances the Welch test and the multiple Welch test were used. P-values lower than 0.05 were judged to be significant.

Results

1. Clinical biochemical/pathological evaluation

The indicators for the liver function showed some changes with age: blood alanine aminotransferase decreased from 40.0 ± 1.4 I.U./l (mean ± SEM) for the 7-month-old animals to 31.0 ± 1.1 I.U./l for the 34-month-old and mean plasma albumin in the different age groups varied between 49.1 ± 4.5 mg/l and 23.4 ± 1.8 mg/l. The indicators for the kidney function only showed an age-related decrease in ability to reabsorb water (osmolality decreased from 1985 ± 65 mOsm/l to 850 ± 170 mOsm/l over the whole age range, 24 hour urine production increased from 9.8 ± 0.7 ml to 21.2 ± 2.4 ml). In the control groups minor differences between the different groups were observed (body weight of the different groups varied between 286 ± 6 g and 338 ± 7 g, osmolality between 1571 ± 89 mOsm/l and 2260 ± 160 mOsm/l and blood urea nitrogen between 7.2 ± 0.1 mmol/l and 10.4 ± 0.5 mmol/l).
The pathological evaluation showed all animals to be healthy.

2. Pharmacokinetic evaluation

The values of the pharmacokinetic parameters are summarized in table 1.
In the 'pseudo'-longitudinal ageing study only minor variation was observed in the values of the clearance. Volume of distribution was found to be increased in the 34-month-old animals and also a tendency towards an increased elimination half-life was observed in this age group.
In the control groups very similar observations were made, with no significant changes in the clearance between different groups and an increase in volume of distribution in the control group studied at 34 months.

Parameters related to the distribution of phenobarbital between different age groups are summarized in table 2. Generally, only minor differences in plasma protein binding and the distribution ratios between plasma, cerebrospinal fluid and brain tissue were observed.

Table 1. *The influence of ageing on the pharmacokinetic parameters of phenobarbital in rats following an intravenous bolus dose of 20 mg/kg.*

age (month)	7	14	21	28	34
number of animals	8	8	8	8	5
total body clearance (ml/h.kg)	37.1 ± 1.9	41.7 ± 1.7	44.9 ± 2.5	37.2 ± 2.9	33.9 ± 2.8
volume of distribution (ml/kg)	497 ± 14	550 ± 37	463 ± 22	534 ± 29	645 ± 30[a,c]
elimination half-life (h)	9.4 ± 0.4	9.2 ± 0.5	7.2 ± 0.1[a]	10.6 ± 1.2	13.5 ± 1.1[c]

control group	c7[A]	c14[B]	c21	c28	c34
number of animals	8	8	8	8	8
total body clearance (ml/h.kg)	37.1 ± 1.9	47.1 ± 2.9	46.9 ± 3.5	47.7 ± 1.8[e]	39.8 ± 1.6
volume of distribution (ml/kg)	497 ± 14	609 ± 32	528 ± 18	597 ± 49	633 ± 17[e,g]
elimination half-life (h)	9.4 ± 0.4	9.2 ± 0.7	8.0 ± 0.4	8.7 ± 0.7	11.1 ± 0.9[g]

The results are presented as mean ± SEM

[A] group c7 is the 7-month-old group in the upper part of this table
[B] group c14 is the 7-month-old control group evaluated together with the 14-month-old age group

[a] significantly different from 7-month value, $p < 0.05$
[c] significantly different from 21-month value, $p < 0.05$
[e] significantly different from the value of group c7, $p < 0.05$
[g] significantly different from the value of group c21, $p < 0.05$

3. Pharmacodynamic evaluation

The values of the pharmacodynamic parameters are summarized in table 3. In the 'pseudo'-longitudinal ageing study a significant decrease in the threshold dose of phenobarbital was observed from 264 ± 3 mg/kg (mean ± SEM) at the age of 7 months to 185 ± 5 mg/kg at the age of 34 months. Similar decreases in the threshold concentrations in plasma, plasma ultrafiltrate, cerebrospinal fluid and brain were observed. The control groups showed a minor variation in the threshold dose and the threshold plasma concentration, but this variation did not reflect the decrease in these parameters in the age groups.

Table 2. *The influence of ageing on the relative distribution of phenobarbital at onset of loss of righting reflex.*

age (month)	7	14	21	28	34
number of animals	8	8	8	6	5
conc. csf/conc. plasma free[D]	0.54 ± 0.02 (n=7)	0.58 ± 0.01 (n=7)	0.61 ± 0.01 (n=7)	0.56 ± 0.02 (n=5)	0.44 ± 0.02[b,c,d]
conc. csf/conc. plasma total	0.44 ± 0.01 (n=7)	0.44 ± 0.01 (n=7)	0.49 ± 0.01 (n=7)	0.43 ± 0.02 (n=5)	0.36 ± 0.01[a,b,c]
conc. csf/conc. brain	0.63 ± 0.02 (n=7)	0.66 ± 0.01 (n=7)	0.74 ± 0.04 (n=7)	0.71 ± 0.04 (n=5)	0.62 ± 0.03
plasma protein binding (%)	20.5 ± 1.7	24.2 ± 1.7	19.9 ± 1.0	23.2 ± 1.1	19.4 ± 2.2
conc. brain/conc. plasma free	0.88 ± 0.03	0.89 ± 0.02	0.83 ± 0.03	0.82 ± 0.04	0.72 ± 0.03[b]
conc. brain/conc. plasma total	0.70 ± 0.02	0.66 ± 0.01	0.74 ± 0.03	0.63 ± 0.03	0.58 ± 0.02[a]

control group	c7[A]	c14[B]	c21	c28	c34
number of animals	8	8	8	8	8
conc. csf/conc. plasma free[D]	0.54 ± 0.02 (n=7)	0.58 ± 0.03	0.68 ± 0.04 (n=7)	0.65 ± 0.05 (n=7)	0.59 ± 0.02 (n=7)
conc. csf/conc. plasma total	0.44 ± 0.01 (n=7)	0.46 ± 0.02	0.51 ± 0.01 (n=7)	0.52 ± 0.05 (n=7)	0.49 ± 0.01 (n=7)
conc. csf/conc. brain	0.63 ± 0.02 (n=7)	0.64 ± 0.03	0.72 ± 0.03 (n=7)	0.76 ± 0.02[e,f] (n=7)	0.70 ± 0.03 (n=7)
plasma protein binding (%)	20.5 ± 1.7	20.5 ± 1.0	23.8 ± 3.1	21.2 ± 3.7	17.5 ± 1.6
conc. brain/conc. plasma free	0.88 ± 0.03	0.91 ± 0.02	0.94 ± 0.06	0.87 ± 0.05	0.85 ± 0.03
conc. brain/conc. plasma total	0.70 ± 0.02	0.73 ± 0.01	0.70 ± 0.01	0.68 ± 0.05	0.70 ± 0.04

The results are presented as mean ± SEM

[A] group c7 is the 7-month-old group in the upper part of this table
[B] group c14 is the 7-month-old control group evaluated together with the 14-month-old age group
[D] conc. = concentration; csf = cerebrospinal fluid

[a] significantly different from 7-month value, $p < 0.05$
[b] significantly different from 14-month value, $p < 0.05$
[c] significantly different from 21-month value, $p < 0.05$
[d] significantly different from 28-month value, $p < 0.05$
[e] significantly different from the value of group c7, $p < 0.05$
[f] significantly different from the value of group c14, $p < 0.05$

Table 3. *The influence of ageing on dose and concentrations of phenobarbital at onset of loss of righting reflex during an intravenous infusion at a rate of 3 mg/min.*

age (month)	7	14	21	28	34
number of animals	8	8	8	8	5
threshold dose (mg/kg)	264 ± 3	239 ± 5[a]	224 ± 5[a]	206 ± 9[a,b]	185 ± 5[a,b,c]
plasma concentration (mg/l)	425 ± 9	419 ± 14	357 ± 11[a,b]	356 ± 8[a,b]	316 ± 6[a,b,d]
plasma ultrafiltrate concentration (mg/l)	339 ± 12	313 ± 5	286 ± 8[a]	273 ± 8[a,b]	255 ± 3[a,b,c]
cerebrospinal fluid concentration (mg/l)	187 ± 6 (n=7)	186 ± 6 (n=7)	177 ± 6 (n=7)	154 ± 10 (n=5)	113 ± 4[a,b,c,d]
brain concentration (mg/kg)	296 ± 7	280 ± 7	237 ± 10[a,b]	222 ± 9[a,b]	184 ± 8[a,b,c]

control group	c7[A]	c14[B]	c21	c28	c34
number of animals	8	8	8	8	8
threshold dose (mg/kg)	264 ± 3	258 ± 7	250 ± 8[e]	273 ± 4[g]	258 ± 4
plasma concentration (mg/l)	425 ± 9	420 ± 9	384 ± 5[e,f]	421 ± 16[g]	380 ± 9
plasma ultrafiltrate concentration (mg/l)	339 ± 12	334 ± 7	293 ± 14	329 ± 9	313 ± 6
cerebrospinal fluid concentration (mg/l)	187 ± 6 (n=7)	194 ± 9	193 ± 4 (n=7)	213 ± 10 (n=7)	184 ± 4 (n=7)
brain concentration (mg/kg)	296 ± 7	305 ± 9	270 ± 5	281 ± 8	266 ± 10

The results are presented as mean ± SEM

[A] *group c7 is the 7-month-old group in the upper part of this table*
[B] group c14 is the 7-month-old control group evaluated together with the 14-month-old age group

[a] significantly different from 7-month value, $p < 0.05$
[b] significantly different from 14-month value, $p < 0.05$
[c] significantly different from 21-month value, $p < 0.05$
[d] significantly different from 28-month value, $p < 0.05$
[e] significantly different from the value of group c7, $p < 0.05$
[f] significantly different from the value of group c14, $p < 0.05$
[g] significantly different from the value of group c21, $p < 0.05$

Discussion

In the present study a 'pseudo'-longitudinal study design was applied in order to verify that the age-related increase in brain sensitivity for phenobarbital, measured in a previous study with a cross-sectional study design (Stijnen et al., in press; chapter 4), were indeed the result of the ageing process and not of cohort effects.

In the 'pseudo'-longitudinal design one group of animals, born within a period of two weeks, was reserved for this study and at five different ages one subgroup was investigated. At the start of the study the animals were randomly assigned to the subgroups. Together with every subgroup, a control group of young animals was included in the investigations in order to try to detect possible differences caused by the experimental conditions at the time of the year during which the experiments were performed or possible cohort differences.

It is generally accepted that in pharmacological investigations the adaption of a longitudinal study design can offer significant advantages (Rowe, 1977). Especially, the reduction in variability and the increased power to detect statistically significant differences in longitudinal studies, in which every animal is repeatedly studied at different ages, are important considerations in this respect. Also in ageing research these longitudinal study designs have been used. It should be realized however that apart from certain advantages there are also limitations associated with longitudinal studies, especially in ageing research. From a practical point of view it is important that cannulas (used for infusion of drugs or sampling of blood or cerebrospinal fluid) and electrodes (necessary for pharmacodynamic investigations), rarely remain patent over the lifetime of the animal.

A more fundamental problem is that in ageing studies it is impossible to exclude the role of carry-over effects from one experiment to the other, since rank order of the experiments cannot be randomized. Moreover, it has been demonstrated that in addition to ageing the occurrence of concurrent disease may markedly influence the outcome of pharmacological investigations (Stijnen et al., submitted; chapter 6). Therefore, a post mortem tissue examination, performed immediately after the experiment is essential for the interpretation of the results. Obviously such an examination cannot be conducted in a longitudinal study within one animal.

These considerations lead us to conduct the present study on the basis of an

adapted 'pseudo'-longitudinal design, as described before. In this design the advantage of a longitudinal study concerning the reduction of the variability is not valid. However, it should be considered that the interindividual variability in the pharmacodynamic parameter (threshold phenobarbital concentration at onset of loss of righting reflex) is small (Levy, 1985).

Using the 'pseudo'-longitudinal design, a decrease in the threshold dose of phenobarbital needed for onset of loss of righting reflex was found during ageing, without important changes in the pharmacokinetics. The concentration in cerebrospinal fluid at onset of loss of righting reflex decreased during ageing. Together with the fact that the relative distribution of phenobarbital at onset of loss of righting reflex did not show important changes (table 2), this shows an increased brain sensitivity during ageing (Danhof and Levy, 1984), which is the main reason for the decreased dose requirement of phenobarbital during ageing.

The values of the threshold dose of phenobarbital and the concentration in cerebrospinal fluid at onset of loss of righting reflex in the control animals showed some variation in time, but did not decrease to the same extent as the age groups (table 3). This implied that the decrease in the threshold dose with increasing age is not caused by the different times at which the experiments for the different age groups were performed and that the animal model does not show a tendency to a higher sensitivity for phenobarbital.

Both the decrease in phenobarbital dose requirement for onset of loss of righting reflex and the increase in brain sensitivity measured in the present 'pseudo'-longitudinal study design were comparable to those found in the cross-sectional study (Stijnen et al., in press; chapter 4). These findings confirmed the results of the cross-sectional study, thereby excluding the interference of cohort differences.

The fact that the results of the 'pseudo'-longitudinal study confirm those of the cross-sectional set up justifies the use of a cross-sectional study design in these kind of experiments. In addition, the 'pseudo'-longitudinal design offers a good alternative for the common longitudinal and the cross-sectional design, by overcoming disadvantages of both these designs.

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References

Bender, A.D.: Drug sensitivity in the elderly. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson eds., The Macmillan Press Ltd., 1979, pp 147-153.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Thiopentone sensitivity in young and elderly women. Br. J. Anaesth. 55: 33-40, 1983.

Curcio, C.A., McNelly, N.A. and Hinds, J.W.: Variation in longevity of rats: evidence for a systematic increase in lifespan over time. Exp. Aging Res. 10: 137-140, 1984.

Danhof, M. and Levy, G.: Kinetics of drug action in disease states. I. Effect of infusing rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 229: 44-50, 1984.

Gornall, A.C., Bardawill, C.J. and David, M.M.: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 11: 751-766, 1949.

Kaiko, R.F., Wallenstein, S.L., Rogers, A.G., Grabinski, P.Y. and Houde, R.W.: Narcotics in the elderly. Med. Clin. North America 66: 1079-1089, 1982.

Lesser, G., Deutsch, T.S. and Markofsky, J.: Aging in the rat: longitudinal and cross-sectional study of body composition. Am. J. Physiol. 225, 1472-1478, 1973.

Levy, G.: Variability in animal and human pharmacodynamic studies. in: Variability in drug therapy: Description, estimation and control. Rowland M., Sheiner L.B. and Steimer J-L., eds., Raven Press, New York, 1985, pp 125-138.

Martinek, R.G.: Evaluation of a dye-binding method for determination of serum albumin. Clin. Chem. 2: 441-447, 1965.

Miller R.: Anesthesia for the aged: Some pharmacokinetic and pharmacodynamic considerations. The Mount Sinai J. Med. 54: 305-307, 1987.

Mos, J. and Hollander, C.F.: Analysis of survival data on aging rat cohorts: Pitfalls and some practical considerations. Mech. Ageing Dev. 38: 89-105, 1987.

Ness A.T., Dickerson, H.C. and Pastewka, J.V.: The determination of human serum albumin by

its specific binding to the anionic dye, 2-(4'-hydroxybenzeneazo) benzoic acid. Clin. Chim. Acta 12: 532-541, 1965.

Roberts, P., Turnbull, M.J. and Winterburn, A.: Diurnal variation in sensitivity to and metabolism of barbiturate in the rat: lack of correlation between *in vivo* and *in vitro* findings. Eur. J. Pharmacol. 12: 375-377, 1970.

Rowe, J.W.: Clinical research on aging: strategies and directions. New Engl. J. Med. 297: 1332-1336, 1977.

Schlettwein-Gsell, D.: Survival curves of an old rat colony. Gerontologia 16: 111-115, 1970.

Stanski, D.R. and Maitre P.O.: Population pharmacokinetics and pharmacodynamics of thiopental: The effect of age revisited. Anesthesiology 72: 412-422, 1990.

Stijnen, A.M., Danhof, M. and Van Bezooijen, C.F.A.: Increased sensitivity to the anaesthetic effect of phenobarbital in ageing BN/BiRij rats. J. Pharmacol. Exp. Ther., in press.

Stijnen, A.M., Velders-van de Voort, S.H., Zurcher, C., Danhof, M. and Van Bezooijen, C.F.A.: The influence of concomitant disease on age-related changes in the pharmacokinetic-pharmacodynamic relationship of heptabarbital in rats. Submitted for publication.

Chapter 6

THE INFLUENCE OF CONCURRENT DISEASE ON AGE-RELATED CHANGES IN THE PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP OF HEPTABARBITAL IN RATS

A.M. Stijnen[1,2], S.H. Velders-van de Voort[1,2], C. Zurcher[2], M. Danhof[1] and C.F.A. van Bezooijen[2]

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, Leiden, The Netherlands
[2]TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands

submitted for publication

Summary

The purpose of this investigation was to determine the relevance of an extensive pathological investigation in studies on the influence of ageing on the pharmacokinetic-pharmacodynamic relationship of drugs acting on the central nervous system. The study was conducted in 38 BN/BiRij rats of ages between 7 and 31 months. Heptabarbital was chosen as a model drug and its pharmacokinetics and pharmacodynamics were determined following an intravenous bolus dose of 85 mg/kg. The pathological evaluation was performed by an independent pathologist, on the basis of post-mortem tissue examination and additional clinical biochemical data.
With increasing age an increase in the incidence of serious clinically relevant pathology was observed with 5 out of 10 animals affected in the 31-month-old animals.

In the group as a whole, a significant increase in the duration of the pharmacological response (loss of righting reflex) was observed from 23.3 ± 2.6 minutes (mean ± SEM) in the 7-month-old animals to 49.5 ± 6.0 minutes at the age of 31 months. This difference was primarily due to a difference in the pharmacokinetics (the mean residence time tended to increase from 26.1 ± 1.7 minutes to 40.4 ± 5.6 minutes) with no apparent changes in pharmacodynamics. When only the healthy animals were considered however, no important changes in pharmacokinetics were observed, whereas the pharmacodynamics appeared to show an increase in brain sensitivity between the ages of 24 and 31 months (the heptabarbital cerebrospinal fluid concentration at offset of loss of righting reflex being decreased from 20.0 ± 1.1 mg/l to 14.1 ± 1.0 mg/l).

A more detailed examination of the role of concurrent pathology was performed on the 31-month-old group. This analysis revealed a profound effect of disease in both the pharmacokinetics and the pharmacodynamics: diseased animals exhibited a significantly lower clearance and a tendency towards a lower brain sensitivity to heptabarbital compared to their healthy counter parts.

On the basis of these results it is concluded that the presence of concurrent disease can have important implications for the interpretation of studies on the influence of ageing on pharmacokinetic-pharmacodynamic relationships.

Introduction

Ageing is known to be associated with a relatively high incidence of pathology (Brody and Schneider, 1986). In order to be able to investigate age-related changes *per se* excluding the influence of concurrent pathology, admission criteria for gerontological studies in man have been developed (Ligthart et al., 1984). Although it has been demonstrated that experimental animals also develop multiple pathology during ageing (Burek, 1978; Hollander, 1981; Zurcher and Hollander, 1982; Hollander et al., 1990), exclusion of diseased animals from studies on ageing is often not taken into consideration.

The purpose of the present study was to determine the role of concurrent disease in a study concerning the influence of ageing on the pharmacokinetic-pharmacodynamic relationship of heptabarbital in an animal model. Heptabarbital was chosen as a model drug, because it is not a chiral compound and it has a relatively short elimination half-life. Moreover, age-

related pharmacodynamic changes can be expected for this model drug (Stijnen et al., in press; chapter 4). In order to be able to define the health status of the animals under investigation, extensive clinical biochemical and pathological evaluation was performed and animals were judged to be healthy or not by an independent pathologist, who was blinded with regard to the outcome of the pharmacokinetic and pharmacodynamic investigations.

Materials and methods

Animals

Four groups of nine or ten apparently healthy male BN/BiRij rats (TNO IVVO, Leiden, The Netherlands) of different ages (7, 13, 24 and 31 months) were used. The 10, 50 and 90 % survival ages of this strain and sex are 38.1, 31.7 and 22.8 months, respectively. During the period in which the experiments were performed, the rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature was maintained at 22-23 °C, the relative humidity at 60%. The rats were allowed free access to water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands).

Chemicals

Heptabarbital was kindly donated by Ciba-Geigy (Arnhem, The Netherlands).

Animal experiments

1. Clinical biochemical/ pathological evaluation

To determine the health status of the animals, several clinical biochemical indices were measured prior to the pharmacokinetic and the pharmacodynamic evaluation. Blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase and glucose were measured using a Reflotron Autoanalyser (Boehringer, Mannheim, Germany). Total protein concentrations in plasma were determined by means of the

biuret reaction (Gornall et al., 1949), using bovine serum albumin as a standard. Albumin concentrations were measured using 2-(4'-hydroxybenzoic arsene)benzoic acid (Rutstein et al., 1954; Ness et al., 1965; Martinek, 1965). Rat albumin was used as a standard. Urine was collected during 24 hours while the rats were kept in metabolic cages. The volume of the urine was determined and the osmolality was measured using a Digimatic Osmometer 3D II (Advanced Instruments Inc., Mass., USA). The creatinine concentrations in plasma and urine were measured using the Sigma Test Kit No. 555-A (Sigma Chemical Co., St. Louis, MO, USA).
After the pharmacokinetic and pharmacodynamic evaluation, the animals were sacrificed and the liver was weighed; the heart, the lungs, the kidneys, the liver, the brain and macroscopically visible abnormalities in other organs or tissues were evaluated microscopically by a pathologist, who was unaware of the outcome of the pharmacokinetic and pharmacodynamic evaluation. The animals were judged to be diseased if one or more of the following criteria were met: severe inflammatory lesions / lesions with significant necrosis / generalized vascular disease / severe lesions, including tumours, incompatible with normal functioning of heart, lung, liver, kidney or central nervous system / intracranial tumours. In those cases where the clinical relevance of the histopathological lesions was doubtful, abnormal clinical biochemical data were used to decide between healthy or diseased rats.

2. Pharmacokinetic evaluation

85 mg/kg Heptabarbital was administered i.v. via the penal vein under a light halothane anaesthesia. During a period of 180 min after administration 16 blood samples of 120 µl were taken from an incision in the tail.
In order to minimize the influence of a possible diurnal rhythm in brain sensitivity and/ or rate of metabolism (Roberts et al., 1970), both the pharmacokinetic and the pharmacodynamic evaluations were performed between 9:00 A.M. and 1:00 P.M..

3. Pharmacodynamic evaluation

This evaluation was performed one week after the pharmacokinetic evaluation. After intravenous administration of 85 mg/kg heptabarbital the time needed to reach offset of loss of righting reflex (sleeping time) was determined as described previously (Danhof and Levy, 1984). At this point, cerebrospinal fluid, blood and brain tissue were collected. During the

experiment, the body temperature was maintained at 37°C by placing the animals on an isothermal heating-plate.

Drug analysis

The concentrations of heptabarbital in plasma, cerebrospinal fluid and brain tissue were measured by an HPLC-method described by Danhof and Levy (1985). Details on the apparatus used have been described before (Stijnen et al., in press; chapter 4).
Protein binding of heptabarbital was determined by means of ultrafiltration and the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands). The concentrations of heptabarbital in the ultrafiltrate were measured in the same way as that for the plasma samples.

Data analysis

1. Pharmacokinetics
The area under the phenobarbital concentration-time curve (AUC) was calculated using the linear trapezoidal rule. Since it was not possible to determine the terminal half life properly in all animals, due to nonlinearity in the pharmacokinetics, and the concentration of heptabarbital in the last sample was always below the detection limit, the AUC was determined until the detection limit (3 mg/l) was reached for all animals.
The time averaged body clearance was calculated as dose/AUC, the steady state volume of distribution as dose*AUMC/AUC^2 and the mean residence time as AUMC/AUC. The AUMC (area under the concentration * time vs. time curve) was calculated using the linear trapezoidal rule with extrapolation to the detection limit.

2. Statistics
The effect of ageing on the biochemical, pharmacokinetic and pharmacodynamic parameters was statistically tested by one-way analysis of variance with the Student's t-test with Bonferroni correction used to examine differences between age groups. Bartlett's test was used to assess homogeneity of variances. In case of nonhomogeneity of variances the Welch test and the multiple Welch test were used. P-values lower than 0.05 were

judged to be significant.
The influence of disease on the biochemical, pharmacokinetic and pharmacodynamic parameters was evaluated using the Student's t-test or the Welch test, after assessing the homogeneity of variances using the F-test.

Results

1. Clinical biochemical/pathological evaluation
The results of the clinical biochemical indices and some general parameters reflecting the condition of the animal are shown in table 1. Only a few minor changes were found during ageing (a decrease in osmolality and an increase in urine production at the age of 24 months).
At the pathological evaluation a total of eight diseased animals was observed, one 13-month-old, two 24-month-old and five 31-month-old rats. Details about the pathological changes are shown in table 2. Some of the diseased animals showed abnormal values for a few clinical biochemical indices (values were judged abnormal when the deviation from the lowest or the highest value, respectively, of the parameter concerned in the healthy animals in the age group was more than 25%). On the whole however, no significant differences were observed in the mean values of the clinical biochemical indices in healthy and diseased animals (table 3).

2. Pharmacokinetic evaluation
The heptabarbital plasma concentration vs. time profile following an intravenous bolus dose of 85 mg/kg (figure 1) showed a non-linearity in the pharmacokinetics. Therefore, the time averaged body clearance, the steady state volume of distribution and the mean residence time were calculated as pharmacokinetic parameters.
The values of several pharmacokinetic parameters in the different age groups are summarized in figure 2, for both the healthy animals and the diseased ones. The clearance showed a nonsignificant tendency to decrease, with tendency to an increase for the mean residence time in the group as a whole. However, when only the healthy animals were considered these tendencies disappeared. The steady state volume of distribution did not show age- or disease-related changes.

Table 1. *Effect of age on selected clinical biochemical indices.*

age (month)	7	13	24	31
number of animals	9	10	9	10
body weight (g)	300 ± 8	357 ± 6[a]	403 ± 8[a,b]	398 ± 9[a,b]
liver weight (g)	8.7 ± 0.5	9.5 ± 0.3	11.0 ± 0.5[a]	11.2 ± 0.5[a,b]
liver wt/ body wt (%)	2.90 ± 0.14	2.70 ± 0.05	2.71 ± 0.09	2.85 ± 0.10
blood aspartate aminotransferase (I.U./l)	86.9 ± 7.2	85.7 ± 11.0	74.0 ± 5.7	79.7 ± 4.5
blood alanine aminotransferase (I.U./l)	34.1 ± 1.1	43.0 ± 6.7	34.5 ± 1.5	36.2 ± 1.71
plasma albumin (mg/ml)	35.1 ± 0.9	37.3 ± 0.6	34.3 ± 1.0	33.6 ± 1.0
plasma total protein (mg/ml)	82.7 ± 2.3	83.2 ± 2.1	79.9 ± 1.3	83.5 ± 2.6
blood glucose (mmol/l)	4.7 ± 0.3	4.7 ± 0.3	5.1 ± 0.2	5.0 ± 0.5
blood urea nitrogen (mmol/l)	8.3 ± 0.2	8.2 ± 0.2	7.9 ± 0.2	8.7 ± 0.5
creatinine clearance (ml/h.kg)	174 ± 21	200 ± 15	205 ± 24	167 ± 26
urine production (ml/24 hrs)	7.3 ± 0.5	8.7 ± 0.8	9.4 ± 0.4[a]	11.9 ± 1.5
osmolality (mOsm/l)	1660 ± 140	1383 ± 56	1337 ± 70	1075 ± 92[a]

Results are presented as mean ± SEM

[a] significantly different from 7-month value, $p < 0.05$
[b] significantly different from 13-month value, $p < 0.05$

The relative distribution of heptabarbital in plasma, cerebrospinal fluid and brain tissue at offset of loss of righting reflex is shown in table 4 for the healthy animals. All different ratios appeared to be constant during ageing, also when the diseased animals were included (results not shown). The same holds for the protein binding.

Table 5 summarizes the mean values of the pharmacokinetic parameters in the healthy animals (n=5) and the diseased animals (n=5) in the 31-month-old group. The steady state volume of distribution appeared not to be influenced by disease, whereas the clearance and the mean residence time did (only the clearance values of healthy and diseased animals appeared to be statistically different).

Table 2. *Pathological changes and abnormal clinical biochemical indices in animals, judged diseased.*

age (month)	pathology	clinical biochemical parameters
13	severe ulcerative dermatitis with secondary bacterial invasion; purulent panoptithalmitis	low blood glucose, high blood aspartate and alanine aminotransferease
24	meningeal granular cell tumour with compression of adjacent left cerebral cortex	high creatinine clearance
24	malignant adrenal pheochromocytoma with necrosis and lung metastases	
31	highly invasive squamous cell carcinoma of the urinary bladder with extensive necrosis; hydronephrosis, left kidney severe, right kidney moderate	low plasma albumin and total protein
31	multifocal interstitial nephritits with fibrosis; hydronephrosis, left kidney severe, right kidney moderate	low creatinine clearance, low osmolality, high urine production
31	bilateral moderate hydronephrosis; large pituitary adenoma of pars distalis	
31	multifocal haemorrhagic pneumonia	low blood glucose
31	pituitary adenoma of pars distalis	high blood urea nitrogen

3. Pharmacodynamic evaluation

The values for the sleeping time after administration of 85 mg/kg heptabarbital and the concentrations in different compartments at offset of loss of righting reflex are given in figure 3. The sleeping time showed a tendency to increase during ageing, both in the group as a whole ($p < 0.05$) and in the selected healthy animals. The threshold concentration of heptabarbital at offset of loss of righting reflex in plasma, cerebrospinal fluid and brain tissue displayed no important changes during ageing when the diseased animals were included. Considering only the healthy animals however, a decrease in the concentrations between the ages of 24 and 31 months was found.

Table 3. *Biochemical parameters for healthy and diseased 31-month-old animals.*

	healthy	diseased
number of animals	5	5
body weight (g)	398 ± 9	386 ± 30
liver weight (g)	11.9 ± 0.3	10.5 ± 0.8
liver wt/ body wt (%)	2.98 ± 0.06	2.71 ± 0.18
blood aspartate aminotransferase (I.U./l)	75.6 ± 8.7	83.7 ± 2.6
blood alanine aminotransferase (I.U./l)	34.5 ± 2.2	37.9 ± 2.5
plasma albumin (mg/ml)	34.1 ± 0.9	33.1 ± 1.9
plasma total protein (mg/ml)	84.2 ± 2.9	82.8 ± 4.7
blood glucose (mmol/l)	5.2 ± 0.6	4.8 ± 0.7
blood urea nitrogen (mmol/l)	8.1 ± 0.3	9.3 ± 1.0
creatinine clearance (ml/h.kg)	164 ± 46	170 ± 32
urine production (ml/24 hrs)	10.4 ± 2.0	13.4 ± 2.1
osmolality (mOsm/l)	1200 ± 120	950 ± 130

Results are presented as mean ± SEM

Table 5 summarizes the mean values of the pharmacodynamic parameters in the healthy animals (n=5) and the diseased animals (n=5) in the 31-month-old group. The sleeping time appeared to be significantly longer in the diseased animals compared to the healthy ones. The concentrations of heptabarbital at offset of loss of righting reflex in plasma (total and free), cerebrospinal fluid and brain tissue tended to be higher in the diseased animals, but this apparent difference did not reach statistical significance.

Table 4. *The influence of ageing on the relative distribution of heptabarbital at offset of loss of righting reflex in healthy animals.*

age (month)	7	13	24	31
number of animals	9	9	7	5
conc. csf/conc. plasma free[A]	1.13 ± 0.11 (n=8)	0.94 ± 0.07 (n=6)	0.98 ± 0.06 (n=6)	1.10 ± 0.14
conc. csf/conc. plasma total	0.61 ± 0.03 (n=8)	0.56 ± 0.05 (n=6)	0.54 ± 0.02 (n=6)	0.65 ± 0.10
conc. csf/conc. brain	1.09 ± 0.10 (n=7)	0.86 ± 0.04 (n=6)	0.99 ± 0.08 (n=6)	1.02 ± 0.11
plasma protein binding (%)	43.7 ± 3.0	40.8 ± 3.2	44.0 ± 2.1	42.0 ± 2.7
conc. brain/conc. plasma free	1.13 ± 0.12 (n=8)	1.03 ± 0.06	1.00 ± 0.07	1.07 ± 0.05
conc. brain/conc. plasma total	0.61 ± 0.06 (n=8)	0.60 ± 0.04	0.56 ± 0.03	0.62 ± 0.04

Results are presented as mean ± SEM

[A] conc. = concentration; csf = cerebrospinal fluid

Table 5. *Pharmacokinetic and pharmacodynamic parameters for healthy and diseased 31-month-old animals.*

	healthy	diseased
number of animals	5	5
total body clearance (ml/min.kg)	28.7 ± 2.5	16.4 ± 4.0[a]
steady state volume of distribution (ml/kg)	799 ± 64	744 ± 84
mean residence time (min)	28.2 ± 1.9	52.6 ± 8.0[a]
sleeping time (min)	36.4 ± 5.0	62.6 ± 7.2[a]
plasma concentration at offset of LRR (mg/l) [A]	23.3 ± 2.7	40.6 ± 8.9
plasma ultrafiltrate concentration at offset of LRR (mg/l)	13.3 ± 1.2	23.0 ± 5.0
cerebrospinal fluid concentration at offset of LRR (mg/l)	14.1 ± 1.0	19.0 ± 2.9 (n=4)
brain concentration at offset of LRR (mg/kg)	14.2 ± 1.2	21.2 ± 3.6

Results are presented as mean ± SEM

[A] LRR = loss of righting reflex

[a] significantly different from value of the healthy animals, $p < 0.05$

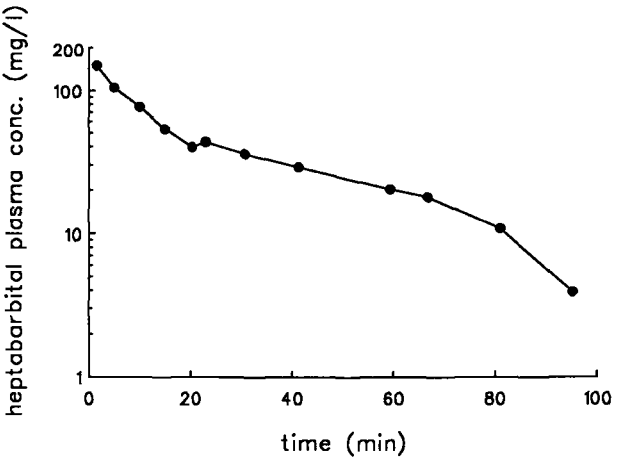

Figure 1. Heptabarbital plasma concentration vs. time profile after administration of an intravenous bolus dose of 85 mg/kg.

Discussion

Ageing is known to be associated with an increasing number of multiple pathological changes (Burek, 1978; Zurcher and Hollander, 1982; Brody and Schneider, 1986; Hollander et al., 1990). Therefore, interpretation of the results of studies on the influence of ageing *per se* may be obscured by underlying diseases (Hijmans et al., 1987; Williams, 1987; Ligthart, 1989; Hollander et al., 1990). This holds true also for pharmacokinetic and pharmacodynamic investigations. Several diseases have been shown to have an influence on the pharmacokinetics and pharmacodynamics of drugs in animals (Kato, 1977; Danhof et al., 1984; Dingemanse et al., 1988b; Hoffman and Levy, 1990) as well as in man (Brater, 1980; Wright and Robson, 1980; Christensen et al., 1983; Fisch et al., 1986; Stanski, 1987; Rambeck et al., 1990).

Thus it appears to be important to separate changes due to ageing itself from effects of complicating disease. One proposed solution could be to exclude all individuals with any known age-related disease. Depending on the diagnostic criteria applied this will result in a more or less severe reduction in the number of so called normally aged individuals (Ligthart, 1989).

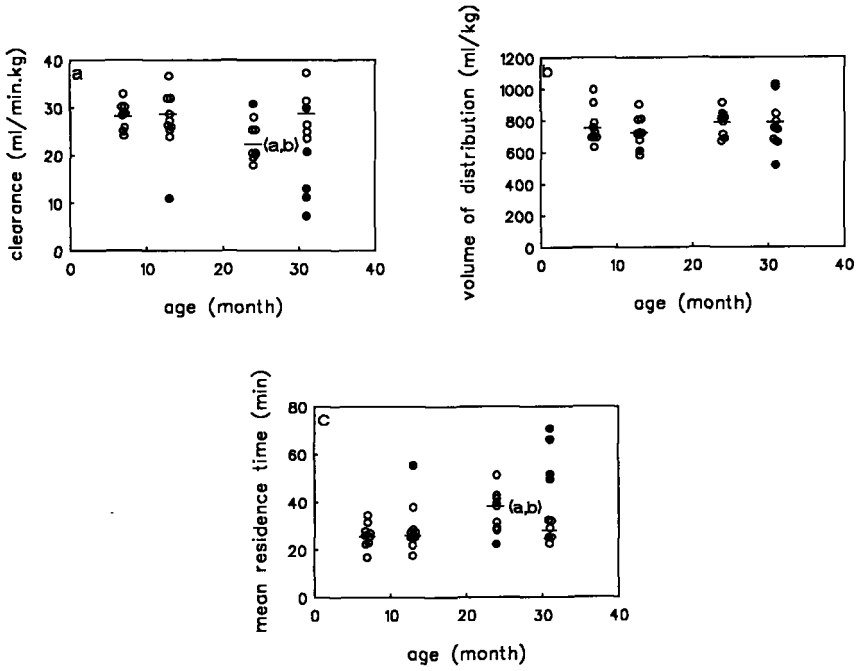

Figure 2. The influence of ageing and concurrent disease on the pharmacokinetic parameters of heptabarbital in rats following an intravenous bolus dose of 85 mg/kg.
o healthy animals; • diseased animals; - mean value of the healthy animals

a. time averaged body clearance
b. steady state volume of distribution
c. mean residence time

$^{(a,b)}$ significantly different from 7- and 13-month value, $p < 0.05$

Consequently, one could have doubts on the representativity of results obtained in such a highly selective part of the ageing population. Another solution could be to exclude not all age-related disease, but only those which most probably will affect the functions to be tested.
In our ageing rat study we chose for not beforehand excluding any animal from testing, but to record whether at necropsy diseases were present which most probably interfered with normal functioning of liver, kidney or brain, being the organs directly involved in the testing procedure (Kato, 1977; Danhof et al., 1984; Dingemanse et al., 1988b; Hoffman and Levy, 1990). Results obtained in all animals were compared with those obtained in rats

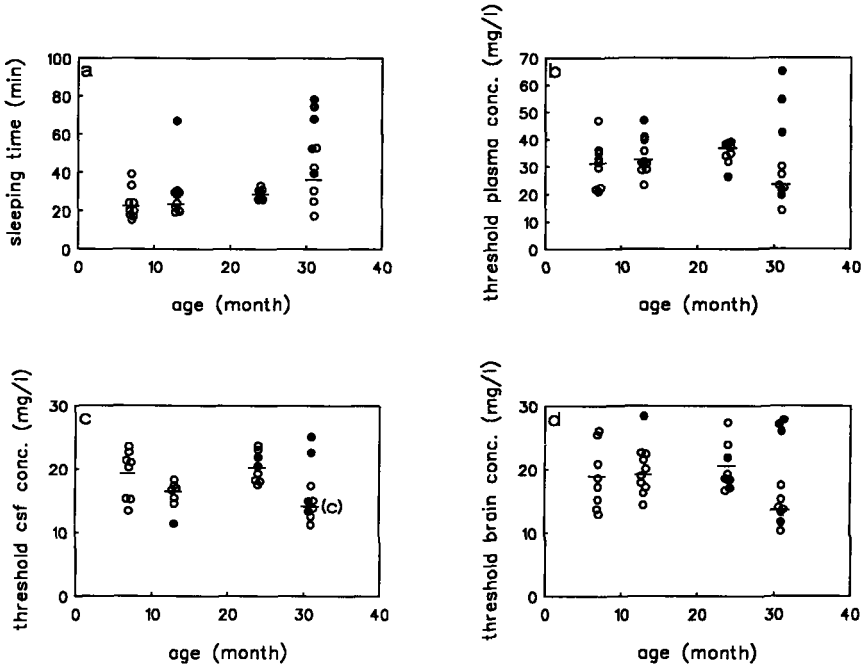

Figure 3. The influence of ageing and concurrent disease on the pharmacodynamic parameters of heptabarbital in rats following an intravenous bolus dose of 85 mg/kg.
o healthy animals; ● diseased animals; - mean value of the healthy animals

a. sleeping time
b. plasma concentration of heptabarbital at offset of loss of righting reflex
c. cerebrospinal fluid concentration of heptabarbital at offset of loss of righting reflex
d. brain concentration of heptabarbital at offset of loss of righting reflex

(c) significantly different from 24-month value, $p < 0.05$

without diseases, which may be expected to interfere. Judgement of whether or not a disease process should be recorded as probably interfering depended on rather subjective criteria, as no solid data on the relationship between the severity of pathological lesions and pharmacokinetic and pharmacodynamic drug testing are known. As a rule those lesions were noted which affected a major part of the critical organs or which were indicative of generalized disease. In those cases where doubt existed on the clinical significance of the histological lesions, the clinical biochemical data were used

to reach a conclusion.

On the basis of the pathology data, eight animals were found to suffer from one or more severe diseases in the present study. In order to be able to study both the effect of ageing and the effect of concurrent disease on the pharmacokinetic-pharmacodynamic relationship of heptabarbital, the pharmacokinetic and pharmacodynamic parameters were evaluated separately for data obtained in all animals (healthy and diseased) and for those obtained only in the healthy animals. In addition, a comparison was made between healthy and diseased animals in the 31-month-old group.
Considering all animals (both healthy and diseased), the sleeping time tended to increase with increasing age (figure 3). The pharmacokinetic parameters mean residence time and clearance showed a tendency to increase and decrease, respectively, with increasing age (figure 2), whereas no pharmacodynamic changes were observed (figure 3). These changes in pharmacokinetics explain (at least in part) the increasing sleeping time.
After exclusion of the diseased rats, again a tendency for an increasing sleeping time was found during ageing. The pharmacokinetics did not show important changes (only the 24-month-old animals exhibited a deviating value; see figure 2).
The concentrations of heptabarbital in plasma, cerebrospinal fluid and brain tissue at offset of loss of righting reflex showed about the same pattern during ageing (figure 3): a decrease in the concentrations was seen between the ages of 24 and 31 months. The cerebrospinal fluid and the brain tissue are pharmacokinetically indistinguishable from the site of action of heptabarbital (Dingemanse et al., 1988). At offset of loss of righting reflex a distribution equilibrium is reached, so plasma is also in equilibrium with the site of action at that point (Dingemanse et al., 1988a). On the basis of these facts and since no differences between the four age groups were found in the ratios between the concentrations at the different sites (table 4), it can be concluded from the decrease in threshold concentration between the ages of 24 and 31 months that the sensitivity of the brain for the anaesthetic effect of heptabarbital increases between these two ages. Thus for the healthy animals this increasing sensitivity mainly explains the longer sleeping time.
The difference in pharmacokinetic and pharmacodynamic changes can be further illustrated on comparison of the five healthy and the five diseased 31-month-old rats. The diseased animals showed longer sleeping times, the

clearance was lower resulting in a tendency to a higher mean residence time and the brain sensitivity tended to be lower compared to the healthy animals. Because of the wide range of values (especially for the diseased animals), not all differences between diseased and healthy rats reached statistical significance.
Exclusion of the diseased animals appeared to be very important. If this aspect would not have been taken into consideration, the conclusion from this study would have been that the pharmacokinetics of heptabarbital changed with age, while the pharmacodynamics did not. By exclusion of the diseased animals, almost the opposite appeared to be true.

All animals suffering from renal diseases showed pharmacokinetic changes. Two out of the three animals displaying hydronephrosis showed pharmacodynamic differences. This impact of renal disease on pharmacokinetics and pharmacodynamics of individual animals is in agreement with findings reported in literature (Wright and Robson, 1980, Danhof et al., 1984, Dingemanse et al., 1988b).
Both animals suffering from pituitary gland tumours showed deviations in the pharmacodynamics (in agreement with data reported by Guthrie et al. (1987)), whereas the meningeal tumour invading into the brain in one 24-month-old animal did not have any effect.

In most articles on ageing no attention is being paid to the health status of the experimental animals. In some reports, however, several clinical biochemical indices for liver and renal function are presented. While clinical biochemical data are regularly gathered during chronic toxicity testing (Hollander et al., 1990), reports on the correlation between such data and age-related pathology in individual cases however are scarce. In one of these studies (Zurcher et al., 1982) no relation between common age-related liver lesions such as cystic cholemic fibrosis, foci and areas and clinical biochemical data for liver function could be established. Concerning kidney functioning, studies directly relating the severity of progressive renal glomerulosclerosis and renal functional parameters in individual cases are lacking. It is especially such data which are required to classify individual animals in a separate diseased animal group.
Considering the results in the present study, the relevance of measuring only clinical biochemical indices in ageing studies is doubtful. Our data show that

not all animals with severe pathology at necropsy and deviating pharmacokinetics or pharmacodynamics could be recognized by the presence of abnormal clinical biochemical indices (table 2). By comparison of the clinical biochemical indices for the five healthy and the five diseased 31-month-old rats (table 3) no significant differences were observed. This stresses the importance of an extensive pathological evaluation in studying pharmacological aspects of physiological ageing.

From the findings of this study it can be concluded that the sleeping time following a 85 mg/kg intravenous bolus dose of heptabarbital increased during ageing in the total group of nonselected male BN/BiRij rats, caused by age-related changes in the pharmacokinetics. When only the healthy animals were considered, the decrease appeared to be mainly caused by an increased brain sensitivity, indicating that the presence or absence of concurrent disease can have important implications for the interpretation of the results of this kind of studies.

The results of this study may have important implications for longitudinal studies, in which each animal is investigated several times during their lifetime, because an extensive pathological evaluation shortly after the investigations is not possible in these studies.

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References

Brater, D.C.: The pharmacological role of the kidney. Drugs 19: 31-48, 1980.

Brody, J.A. and Schneider, E.L.: Diseases and disorders of aging: an hypothesis. J. Chron. Dis. 39: 871-876, 1986.

Burek, J.D.: Pathology of ageing rats. A morphological and experimental study of the age-associated lesions in aging BN/Bi, Wag/Rij and (WAG x BN) F_1 rats. CRC-Press Inc., West Palm Beach, Florida, 1978.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Pharmacokinetics and pharmacodynamics of thiopental in patients undergoing renal transplantation. Acta Anaesthesiol. Scand. 27: 513-518, 1983.

Danhof, M. and Levy G.: Kinetics of drug action in disease states. I. Effect of infusing rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 229: 44-50, 1984.

Danhof, M., Hisoaka, M. and Levy G.: Kinetics of drug action in disease states. II. Effect of experimental renal dysfunction on phenobarbital concentrations in rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 230: 627-631, 1984.

Danhof, M. and Levy, G.: Kinetics of drug action in disease states. V Acute effect of urea infusion on phenobarbital concentrations in rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 232: 430-434, 1985.

Dingemanse, J., Thomassen, D., Mentink B.H. and Danhof M.: Strategy to assess the role of (inter)active metabolites in pharmacodynamic studies in-vivo: a model study with heptabarbital. J. Pharm. Pharmacol. 40: 552-557, 1988.

Dingemanse, J., Hutson, P.H., Langemeijer, M.W.E., Curzon, G. and Danhof, M.: Application of serial sampling of cerebrospinal fluid in pharmacodynamic studies with a drug active in the CNS: Heptabarbital concentrations at onset and offset of loss of righting reflex in rats. Neuropharmacol. 27: 467-474, 1988a.

Dingemanse, J., Polhuijs, M. and Danhof, M.: Altered pharmacokinetic-pharmacodynamic relationship of heptabarbital in experimental renal failure in rats. J. Pharmacol. Exp. Ther. 246, 371-376, 1988b.

Fisch, H.U., Baktir, G., Karlaganis, G. and Bircher, J.: Excessive effects of benzodiazepines in patients with cirrhosis of the liver: A pharmacodynamic or a pharmacokinetic problem? Pharmacopsychiat. 19, 14, 1986.

Gornall, A.C., Bardawill, C.J. and David, M.M.: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 11: 751-766, 1949.

Guthrie, S., Cooper, R.L., Thurman, R. and Linnoila, M.: Pharmacodynamics and pharmacokinetics of ethanol, diazepam and pentobarbital in young and aged rats. Pharmacol. Toxicol. 61: 308-312, 1987.

Hijmans, W., de Greef G.E. and Ligthart, G.J.: Ageing and the immune system in humans. Eur. J. Cell Biol. 44 (S20), 22-23, 1987.

Hoffman, A. and Levy, G.: Kinetics of drug action in disease states. XXXVI Effect of cyclosporine on the pharmacodynamics and pharmacokinetics of a barbiturate (heptabarbital) in rats. J. Pharm. Sci. 79: 19-22, 1990.

Hollander, C.F.: Age-associated pathology in the rat: its consequences for gerontological research. in: Biochemical and morphological aspects of ageing, W.E.G. Müller and J.W. Roden, eds., Akademie der Wissenschaften und der Literatur, Mainz/ Franz Steinder Verlag, Wiesbaden, 1981, pp 220-226.

Hollander, C.F., Delort, P. and Duprat, P.: Toxicology in the older age group. in: Progress in predictive toxicology, D.B. Clayson, I.C. Munro, P. Shubik, J.A. Swenberg, eds., Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 1990, pp 113-142.

Kato, R.: Drug metabolism under pathological and abnormal physiological states in animals and man. Xenobiotica 7: 25-92, 1977.

Ligthart, G.J., Corberand, J.X., Fournier, C., Galanaud, P., Hijmans, W., Kennes, B., Muller-Hermelink, H.K. and Steinmann, C.G.: Admission criteria for immunogerontological studies in man: the SENIEUR protocol. Mech. Ageing Developm. 28, 47-56, 1984.

Ligthart, G.J.: The immune system in human ageing. Necessity of the assessment of health status in gerontological studies. Thesis, University of Leiden, 1989.

Martinek, R.G.: Evaluation of a dye-binding method for determination of serum albumin. Clin. Chem. 11: 444-447, 1965.

Ness, A.T., Dickerson, H.C. and Pastewka, J.V.: The determination of human serum albumin by its specific binding of the anionic dye, 2-(4'-hydroxybenzeneazo)benzoic acid. Clin. Chim. Acta 12: 523-541, 1965.

Rambeck, B., Schnabel, R., May, T., Jürgens, U. and Villagrán, R.: Postmortem serum protein binding and brain concentrations of epileptic drugs in autoptic specimens from 45 epileptic patients. Ther. Drug Monit. 12: 533-540, 1990.

Roberts, P., Turnbull, M.J. and Winterburn, A.: Diurnal variation in sensitivity to and metabolism of barbiturate in the rat: lack of correlation between *in vivo* and *in vitro* findings. Eur. J. Pharmacol. 12: 375-377, 1970.

Rutstein, D.D., Ingenito, E.F. and Reynolds, W.E.: The determination of albumin in human blood plasma and serum. A method based on the interaction of albumin with an anionic dye -2-(4'-hydroxybenzeneazo)benzoic acid. J. Clin. Invest. 33: 211-221, 1954.

Stanski, D.R.: The clinical pharmacology of alfentanil. Eur. J. Anaesthesiol. S1, 3-11, 1987.

Stijnen, A.M., Danhof, M. and Van Bezooijen, C.F.A.: Increased sensitivity to the anaesthetic effect of phenobarbital in ageing BN/BiRij rats. J. Pharmacol. Exp. Ther., in press.

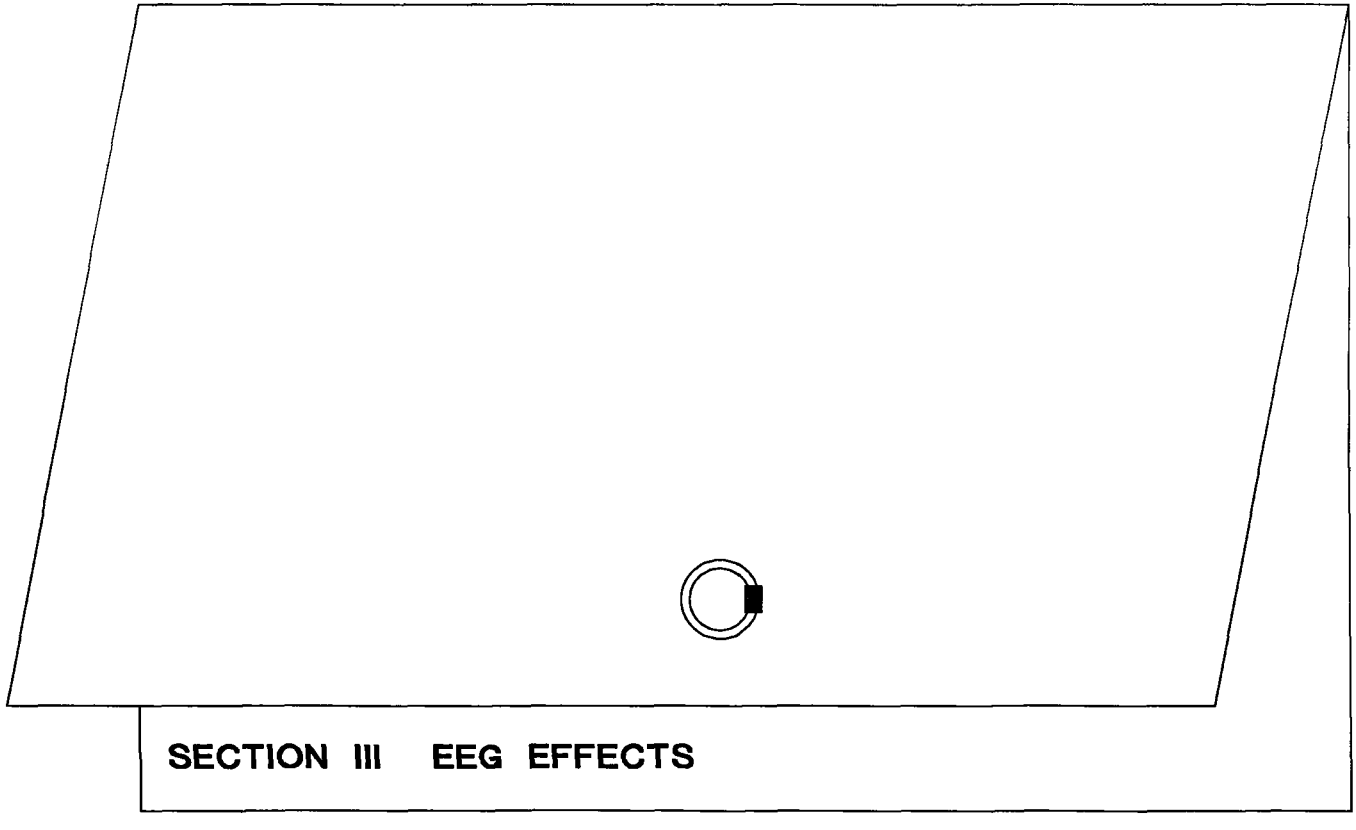
Williams, T.F.: Aging or disease? Clin. Pharmacol. Ther. 42, 663-665, 1987.

Wright, N. and Robson, J.S.: Renal diseases. In: Drug treatment, G.S. Avery, ed., ADIS Press, New York, 1980, 2nd ed., pp. 800-845.

Zurcher, C. and Hollander, C.F.: Multiple pathological changes in aging rat and man. Expl. Biol. Med. 7: 56-62, 1982.

Zurcher, C., van Zwieten, M.J., Solleveld, H.A., van Bezooijen, C.F.A. and Hollander, C.F.:

Possible influence of multiple pathological changes in aging rats on studies of organ aging, with emphasis on the liver. in: Liver and Aging, K. Kitani, ed., Elsevier Biomedical Press, 1982, pp. 19-37.



SECTION III EEG EFFECTS

Chapter 7

UNCHANGED PHARMACODYNAMICS OF HEPTABARBITAL IN AGEING BN/BIRIJ RATS AS DETERMINED BY APERIODIC EEG ANALYSIS

A.M. Stijnen[1,2], A.M. Bergveld[1], J.W. Mandema[1], C.F.A. van Bezooijen[2] and M. Danhof[1]

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, Leiden, The Netherlands
[2]TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands

submitted for publication

Summary

The influence of physiological ageing on the pharmacodynamics of heptabarbital was studied in male BN/BiRij rats (age 4 to 37 months) utilizing the heptabarbital-induced changes in the electroencephalogram (EEG) as a pharmacodynamic measure. Heptabarbital was infused intravenously at a rate of 11 mg/kg.min until isoelectric periods in the EEG of 5 seconds duration. Plasma heptabarbital concentrations and EEG effects were monitored during and after the infusion and subjected to simultaneous pharmacokinetic-pharmacodynamic modelling in order to derive the concentration vs. pharmacological response relationship. Utilizing the total amplitude in the 2.5-30 Hz frequency band as a pharmacodynamic measure, a monophasic effect (decrease) was observed, which could be characterized successfully by the sigmoidal E_{max} model.

In the 4-month-old rats the values of the pharmacodynamic parameters were maximum effect = 69 ± 10 μV/s, heptabarbital concentration at half maximum

effect = 70 ± 3 mg/l and Hill factor = 6.8 ± 1.1. With increasing age no changes in the values of these parameters were observed.
Utilizing the total amplitude in the 0.5-2.5 Hz frequency band, a biphasic effect (increase followed by decrease) was observed. This effect was characterized on the basis of an 'effector - inhibitor model', whereby the biphasic effect is assumed to be the result of one inhibitory mechanism of action at two different effect sites. Utilizing this model, the pharmacodynamics of heptabarbital could also be described on basis of the sigmoidal E_{max} model. The values of the observed pharmacodynamic parameters were in this instance maximum effect = 131 ± 18 μV/s, heptabarbital concentration at half maximum effect = 64 ± 2 mg/l and Hill factor = 3.3 ± 0.2. Again with increasing age, no changes in the values of the pharmacodynamic parameters were observed.
It is concluded on the basis of the observed EEG effects, no important changes in the pharmacodynamics of heptabarbital with ageing seem to occur. This is in contrast to findings with a real measure of anaesthetic effect (loss of righting reflex), which provided evidence for an increased brain sensitivity to heptabarbital and phenobarbital in the same animal model of ageing.

Introduction

It is well established that in the elderly there is a decreased dose requirement of barbiturates for the induction of anaesthesia (Christensen and Andreasen, 1978). The mechanism of this change however, in terms of changes in pharmacokinetics and/or pharmacodynamics is subject to some controversy. Studies in humans, utilizing thiopental-induced changes in the EEG as a measure of pharmacological effect and based on the application of pharmacokinetic-pharmacodynamic modelling concepts, have provided evidence for a change in pharmacokinetics (in particular the central volume of distribution or the rate of distribution to peripheral tissues) as the mechanism of the decreased dose requirement in the elderly (Homer and Stanski, 1985; Stanski and Maitre, 1990). Recent studies in an animal model of ageing on the other hand, utilizing a realistic measure of the anaesthetic effect (loss of righting reflex) have convincingly demonstrated an increase in brain sensitivity with increasing age (Stijnen et al., in press; submitted; chapters 4 and 6). This raises the question to what extent the

pharmacodynamics of the EEG effect show age-related changes comparable with those of the pharmacodynamics of the anaesthetic effect of drugs.
The purpose of the present study was therefore to determine the influence of increasing age on the brain sensitivity to barbiturates in an animal model of ageing, utilizing quantitative EEG parameters as a measure of pharmacological effect. In this study, the same animal model was used as in the previous studies of loss of righting reflex. Heptabarbital was chosen as a model drug because it exhibits relatively rapid elimination kinetics. In addition pharmacokinetic perturbations, which may complicate *in vivo* pharmacodynamic studies, are not likely to occur, since the drug is not a racemic mixture of two enantiomers and active metabolites are formed. Furthermore, acute tolerance development does not occur (Dingemanse et al., 1988, 1988a; Mandema and Danhof, 1990). Heptabarbital has also been used in a previous study on the influence of ageing using loss of righting reflex as the pharmacodynamic measure (Stijnen et al., submitted; chapter 6).

Materials and methods

Animals

Five groups of male BN/BiRij rats (TNO IVVO, Leiden, The Netherlands) of different ages (4, 15, 25, 32 and 37 months) were used. The 10, 50 and 90 % survival ages of this strain are 38.1, 31.7 and 22.8 months, respectively. The animals had *ad libitum* access to water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands). They were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature was maintained at 22-23 °C.

Chemicals

Heptabarbital was kindly donated by Ciba-Geigy (Arnhem, The Netherlands).

Animal experiments

1. Clinical biochemical/pathological evaluation

In order to be able to determine the health status of the animals, several clinical biochemical indices were measured (blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase and glucose, total plasma protein and albumin concentrations, urine production, osmolality and creatinine clearance). Details about the methods used to determine these indices have been described before (Stijnen et al., submitted; chapter 6).

After the pharmacokinetic and pharmacodynamic evaluation, the animals were sacrificed, the liver was weighed, and the heart, the lungs, the kidneys, the liver, the brain and macroscopically visible abnormalities were evaluated microscopically by a pathologist. Details about this evaluation were reported before (Stijnen et al., submitted; chapter 6).

2. Pharmacokinetic/pharmacodynamic evaluation

Cortical EEG electrodes were implanted two weeks before the experiment, using the method described before by Mandema and Danhof (1990). Because the experiments were performed in a restraining cage, the rats were kept in that cage for about 15 minutes each day during the week preceding the experiment. Five hours before the experiments cannulas were implanted in the femoral vein and femoral artery under halothane anaesthesia. All experiments were started around 2.00 P.M. in order to minimize the influence of a possible diurnal rhythm in brain sensitivity, baseline EEG characteristics and/or rate of metabolism (Roberts et al., 1970).

After about 15 minutes baseline EEG recording, heptabarbital was administered via the femoral vein cannula at a rate of 11 mg/kg.min until early burst supression with isoelectric periods of 5 seconds or longer occurred in the EEG. During the infusion and during the period the EEG returned to the baseline pattern, the EEG was continuously measured (Mandema and Danhof, 1990) and blood samples of 120 µl were frequently taken from the femoral artery cannula.

Drug analysis

The concentrations of heptabarbital in plasma, cerebrospinal fluid and brain tissue were measured by an HPLC-method described by Danhof and Levy (1985). The HPLC system (Kratos Analytical Instruments, Ramsey, USA) consisted of a Spectroflow 400 solvent delivery system, a Promis automatic sample injector and a Spectroflow 757 U.V. detector set at 254 nm. A Z-module containing a Radial-Pak C-18 cartridge was used (Waters Associates, Milford, USA). Data processing was performed with a Shimadzu C-R3A reporting integrator.

Data analysis

1. EEG signal analysis

On-line aperiodic analysis was applied for quantification of the EEG data of two leads, F_l-O_l and F_l-C_l (Gregory and Pettus, 1986; Mandema and Danhof, 1990). Thereafter, the total amplitude per second was calculated in the frequency ranges of 0.5-2.5 Hz and 2.5-30 Hz (for one lead, F_l-O_l). These parameters were averaged for periods of 60 seconds or longer (while the concentration was rapidly changing, small periods were chosen) and used as measures of the EEG effect of heptabarbital.

2. Pharmacokinetic-pharmacodynamic modelling

2a. Frequency range of 2.5-30 Hz.

The heptabarbital plasma concentration vs. time profile was described using a two exponential equation for intravenous infusion. The temporal delay (hysteresis) between the plasma concentrations and the EEG effect was estimated using a semi-parametric approach (Fuseau and Sheiner, 1984): after modelling the pharmacokinetics, the hysteresis was solved without assuming a pharmacodynamic model by estimating the link parameter k_{eo} (the first order rate constant, describing the rate of equilibration between the plasma and the effect site). The relationship between the in this way estimated effect compartment concentrations and the above mentioned measures for the EEG effect were characterized by the sigmoidal E_{max} model:

$$E = E_0 + \frac{E_{max}.C^n}{C^n + EC_{50}^n}$$

where E is the observed effect, E_0 is the baseline effect value, E_{max} is the maximum attainable effect, C is the calculated hypothetical effect compartment concentration, EC_{50} the effect compartment concentration causing half the maximum effect and n is a parameter that determined the steepness of the curve.
In 5 out of the total of 43 animals, the estimation for the maximum effect was unphysiologically high. In those animals the complete isoelectric EEG was used as the maximum effect (decrease of total amplitude to zero).

2b. Frequency range of 0.5-2.5 Hz.
In the frequency range of 0.5-2.5 Hz biphasic concentration vs. EEG effect relationships were observed, consisting of an increase in the effect parameters followed by a decrease. The hysteresis was solved parametrically by assuming two effect compartments (estimating the $k_{eo,inhibitor}$ and the $k_{eo,regulator}$), as described by Mandema and Danhof (1990). The biphasic effect is explained by only one inhibitory effect of heptabarbital, exerted directly at the effector (leading to overall inhibition) and exerted on an inhibitor of the effector (leading to overall activation). The concentration vs. effect curves were characterized by the effector-inhibitor model:

$$E = (E_0 + \frac{A_{max}.C_a^n}{C_a^n + EC_{50}^n}) . (1 - \frac{C_i^n}{C_i^n + EC_{50}^n})$$

where A_{max} reflects the hypothetical maximum effect when only desinhibition (inhibiting effect of heptabarbital on the inhibitor, overall effect is activation) would occur and should be considered as a parameter describing the link between the effector and inhibitor. C_a and C_i refer to the concentrations in the two different effect compartments, associated with activation and inhibition, respectively. The model has been described in detail by Mandema and Danhof (1990).

3. Statistics

All calculations for the pharmacokinetic-pharmacodynamic modelling procedures were performed using Siphar (Simed SA, Creteil, France), applying a least squares nonlinear regression algorithm.

The effect of ageing on the biochemical indices, the link model parameter k_{e0} and the pharmacodynamic parameters were statistically tested by one-way analysis of variance and the Student's t-test with Bonferroni correction. In case of nonhomogeneity of variances (assessed by Bartlett's test) the Welch test and the multiple Welch test were used. P-values lower than 0.05 were judged to be significant.

Results

1. Clinical biochemical/pathological evaluation

The results of the clinical biochemical indices are shown in table 1 for all animals. Only the urine osmolality decreased significantly from 1980 ± 140 mOsm/l to 1135 ± 75 mOsm/l.

On the basis of the pathological evaluation six animals were excluded from the pharmacokinetic-pharmacodynamic evaluation (Stijnen et al., submitted; chapter 6). One 4-month-old rat was excluded because of large areas of haemorrhagic liver necrosis, one 15-month-old animal because of large granular lymphocyte leukaemia and a meningeal granular cell tumour and one 25-month-old animal also because of a meningeal granular cell tumour deeply invading into the frontal brain cortex. One 32-month-old rat showed a meningeal osteosarcoma causing compression and necrosis of the cerebral cortex and another one multifocal meningoencephalitis and one 37-month-old animal severe purulent pyelonephritis, cystitis, urethritis, prostatitis and sepsis.

2. Pharmacokinetic/pharmacodynamic evaluation

Figure 1 shows the plasma concentration vs. time profile and the EEG effect vs. time profile in the frequency ranges of 0.5-2.5 Hz and 2.5-30 Hz in one typical rat. In the low frequency range a biphasic response was found, whereas in the frequency range of 2.5-30 Hz a monophasic response was measured.

Table 1. *Effect of age on selected clinical biochemical indices.*

age (month)	4	15	25	32	37
number of animals	8	8	8	6	5
body weight (g)	286 ± 12	351 ± 13[a]	431 ± 12[a,b]	406 ± 15[a]	342 ± 14[c]
liver weight (g)	9.3 ± 0.2	10.9 ± 0.8	12.7 ± 0.7[a]	11.3 ± 1.3	11.6 ± 0.5
liver wt/ body wt (%)	3.29 ± 0.12	3.08 ± 0.13	2.94 ± 0.11	2.74 ± 0.25	3.40 ± 0.11
blood aspartate aminotransferase (I.U./l)	83.0 ± 3.4	78.8 ± 5.2	87 ± 17	70.3 ± 2.3	82.1 ± 4.2
blood alanine aminotransferase (I.U./l)	42.1 ± 2.9	45.3 ± 2.2	42.3 ± 2.5	37.4 ± 1.4	36.6 ± 2.1
plasma albumin (mg/ml)	30.8 ± 2.1	25.3 ± 2.2	21.9 ± 3.0	23.2 ± 2.4	19.5 ± 2.4
plasma total protein (mg/ml)	80.9 ± 2.8	92.1 ± 2.0	89.2 ± 4.0	95.4 ± 4.4	87.0 ± 6.1
blood glucose (mmol/l)	6.7 ± 0.5	6.2 ± 0.3	6.6 ± 0.1	6.1 ± 0.3	5.7 ± 0.3
blood urea nitrogen (mmol/l)	9.1 ± 0.4	8.2 ± 0.1	8.9 ± 0.5	8.4 ± 0.8	7.8 ± 0.3
creatinine clearance (ml/h.kg)	285 ± 51 (*n=7*)	230 ± 41	205 ± 26	203 ± 31	218 ± 19
urine production (ml/24 hrs)	8.3 ± 0.6	7.7 ± 1.1	9.3 ± 1.6	9.4 ± 1.3	11.4 ± 1.3
osmolality (mOsm/l)	1980 ± 140	1550 ± 140	1340 ± 91[a]	1174 ± 71[a]	1135 ± 75[a]

Results are presented as mean ± SEM

[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 15-month value, $p < 0.05$
[c] significantly different from 25-month value, $p < 0.05$

2a. Frequency range of 2.5-30 Hz.

After characterization of the plasma concentration vs. time profile by a two exponential equation for intravenous infusion, the resulting hysteresis loop in the concentration vs. effect profile was solved by estimating the k_{eo}. The estimated steady state concentration vs. effect relationship could be adequately described by the sigmoidal E_{max} model, as is shown in figure 2. Table 2 displays the estimates for the k_{eo} and the pharmacodynamic parameters for the different age groups. None of the parameters showed statistically significant changes during ageing.

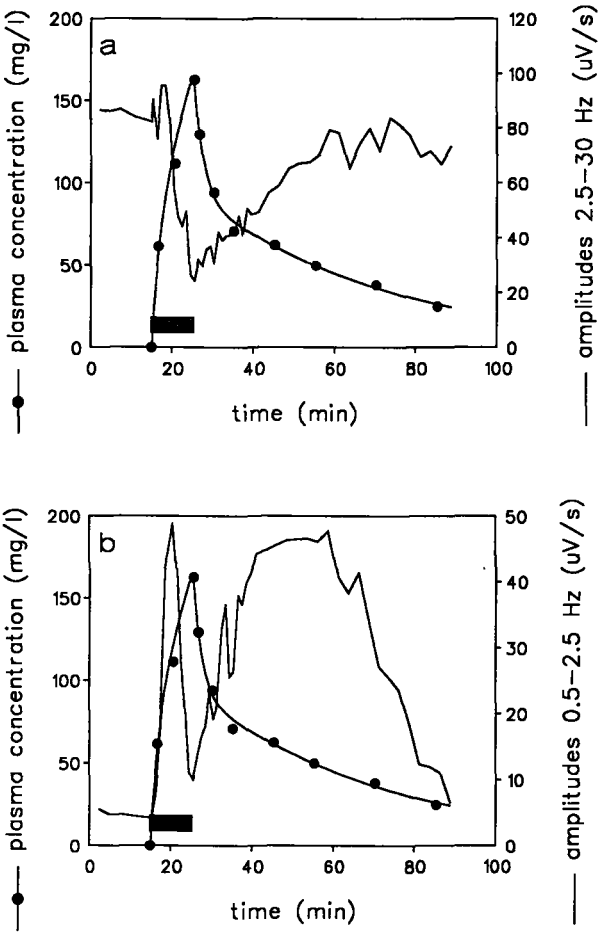


Figure 1. Heptabarbital plasma concentration vs. time profile (the line through the data points represents the characterization of the concentration vs. time relationship by the two exponential equation for infusion) and EEG effect vs. time profiles for a typical rat (4 months of age) using the total amplitude as the descriptor of the EEG effect in the frequency ranges of 2.5-30 Hz (fig. 1a) and 0.5-2.5 Hz (fig. 1b). The solid bar represents the duration of the infusion.

2b. Frequency range of 0.5-2.5 Hz.

Table 3 shows the estimates for the k_{eo} and the pharmacodynamic parameters for the different age groups. No statistically significant age-related changes were observed.

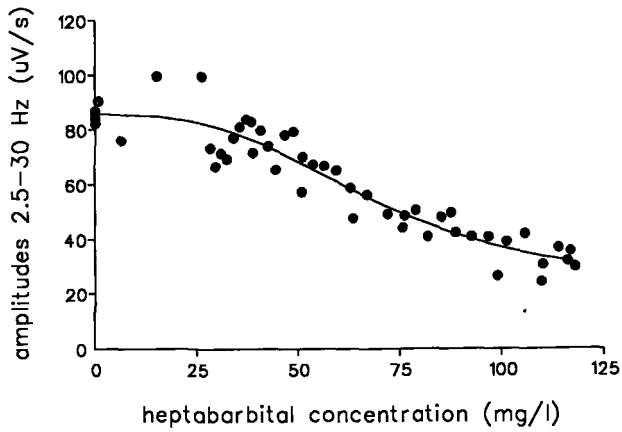


Figure 2. Effect compartment heptabarbital concentration vs. EEG effect relationship for a typical rat (4 months of age) using the total amplitude in the frequency range of 2.5-30 Hz as the descriptor of the EEG effect. The solid line through the data points represents the characterization of the curve by the sigmoidal E_{max} model.

Discussion

For quantitation of pharmacological effects on the central nervous system, drug-induced changes in the EEG can be a useful tool, since they may reflect objective, sensitive and continuous measures of the drug effect, which are obtainable in both animals and humans in a comparable way (Mandema and Danhof, 1990).

In the present study the heptabarbital-induced changes in the EEG were measured in the frequency ranges of 0.5-2.5 Hz and 2.5-30 Hz. These frequency ranges were chosen on the basis of the findings of Mandema and Danhof (1990), who investigated the five 'physiological' frequency ranges. Since four of the five ranges yielded comparable EEG changes, those four ranges were combined to the 2.5-30 Hz range in our study. An advantage of combining the ranges is the derivation of a more stable predictor of the drug effect, since the drug independent variation in the EEG parameters is smaller when a larger frequency range is used.

Table 2. *Influence of age on the link parameter and pharmacodynamic parameter estimates of the characterization of the concentration vs. EEG effect relationships in the frequency range of 2.5-30 Hz, using the amplitude (μV/s) as descriptor of the EEG effect.*

age (month)	4	15	25	32	37
number of animals	7	7	7	4	4
k_{eo} (/min)[A]	0.43 ± 0.17	0.40 ± 0.14	0.24 ± 0.04	0.16 ± 0.03	0.65 ± 0.45
baseline effect value (μV/s)	113 ± 8	123 ± 8	102 ± 6	99 ± 4	96 ± 2
maximum effect (μV/s)	69 ± 10	86 ± 12	70 ± 3	71 ± 4	81 ± 9
EC_{50} (mg/l)[A]	70 ± 3	67 ± 8	59 ± 2	64 ± 2	65 ± 4
shape factor[A]	6.8 ± 1.1	5.2 ± 1.1	5.8 ± 1.0	5.2 ± 0.7	4.6 ± 1.4

Results are presented as mean ± SEM

[A] k_{eo}: first order rate constant, describing the rate of equilibration between the plasma and the effect site
EC_{50}: effect compartment concentration causing half the maximum effect
shape factor: power function that determines the shape of the curve

Table 3. *Influence of age on the link parameter and pharmacodynamic parameter estimates of the characterization of the concentration vs. EEG effect relationships in the frequency range of 0.5-2.5 Hz, using the amplitudes (μV/s) as the descriptor of the EEG effect.*

age (month)	4	15	25	32	37
number of animals	7	7	7	4	4
$k_{eo,inhibitor}$ (/min)[A]	0.32 ± 0.06	0.25 ± 0.02	0.33 ± 0.05	0.21 ± 0.04	0.30 ± 0.11
$k_{eo,regulator}$ (/min)[A]	0.38 ± 0.09	0.23 ± 0.02	0.51 ± 0.09	0.23 ± 0.09	0.70 ± 0.55
baseline effect value (μV/s)	7.8 ± 1.1	7.6 ± 0.7	7.5 ± 0.6	6.4 ± 0.5	9.0 ± 2.6
maximum effect (μV/s)	131 ± 18	124 ± 9	102 ± 8	95 ± 12	115 ± 24
EC_{50} (mg/l)[A]	64 ± 2	58 ± 4	59 ± 2	62 ± 4	54 ± 4
shape factor[A]	3.3 ± 0.2	3.5 ± 0.3	3.7 ± 0.4	3.7 ± 0.7	4.0 ± 0.1

Results are presented as mean ± SEM

[A] k_{eo}: first order rate constant, describing the rate of equilibration between the plasma and the effect site
EC_{50}: effect compartment concentration causing half the maximum effect
shape factor: power function that determines the shape of the curve

An important aspect concerns the constant baseline effect values for the different age groups in both frequency ranges. In addition to the quantification of the baseline effect value, the baseline EEG was examined more qualitatively in the different age groups. The relative contribution of the total number of waves, the amplitude, the amplitude/waves and the power (square amplitude) in the 'physiological' frequency ranges (delta, 0.5-2.5 Hz; theta, 2.5-7.5 Hz; alpha, 7.5-11.5 Hz and beta, 11.5-30 Hz) to the total of these four parameters in the whole frequency range (0.5-30 Hz) was evaluated and resulted in an independence of age (results not shown).
The sigmoidal E_{max} parameters in the frequency range of 2.5-30 Hz did not show age-related changes. In a previous study (Stijnen et al., submitted; chapter 6), the protein binding of heptabarbital appeared to be constant over the whole age range. This means that next to the total concentration at half the maximum effect also the free heptabarbital concentration at half the maximum effect is independent of age. It can be concluded that in the 2.5-30 Hz frequency range the sensitivity of the brain to heptabarbital-induced EEG changes is independent of age.

In a previous study concerning the pharmacokinetic-pharmacodynamic relationship of heptabarbital and phenobarbital in ageing BN/BiRij rats, using the offset of loss of righting reflex as a measure for the anaesthetic effect, an age-related increase in the sensitivity to the barbiturates was found (Stijnen et al., in press; chapter 4). Since we did not find age-related changes in this study using the EEG as a measure for the pharmacological effect, the EEG does not seem to be suitable to study the influence of ageing on the pharmacodynamics of the anaesthetic effect of barbiturates. Apparently, when using the EEG a different aspect of the pharmacological profile is measured compared to using the technique of loss of righting reflex, which is of course a real measure of the anaesthetic effect (more or less similar to commonly used clinical methods).

In studies with thiopental in humans (Homer and Stanski, 1985; Stanski and Maitre, 1990) using the EEG as a measure for the pharmacological effect of thiopental, no age-related changes in pharmacodynamics were found. These studies were performed in a partly different way from the present animal study; Fourier analysis was used to calculate the spectral edge (frequency below which 95% of the power, the square amplitude, is located) as the

descriptor of the EEG effect. Although Fourier analysis is a different technique from aperiodic analysis, it is not to be expected that upon applying the techniques in studies on ageing different results on age-related changes would be found (Mandema et al., personal communication). The results of the present animal study are in agreement with these studies in humans on thiopental-induced EEG changes.

Summarizing, it may be concluded that, in rats, using the total amplitude in the 2.5-30 Hz frequency band as a pharmacodynamic measure for barbiturate-induced EEG changes, these EEG changes do not have a predictive value for the anaesthetic effect of barbiturates. Secondly, because both in rats and humans no age-related changes in the pharmacodynamics of barbiturate- induced EEG changes were observed, there may be a parallel between the barbiturate-induced EEG changes in humans and those in rats.

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References

Christensen, J.H. and Andreasen, F.: Individual variation in response to thiopental. Acta Anaesthesiol. Scand. 22: 303-313, 1978.

Danhof, M., and Levy G.: Kinetics of drug action in disease states. V. Acute effect of urea infusion on phenobarbital concentrations in rats at onset of loss of righting reflex. J. Pharmacol. Exp. Ther. 232: 430-434, 1985.

Dingemanse, J., Thomassen, D., Mentink B.H. and Danhof M.: Strategy to assess the role of (inter)active metabolites in pharmacodynamic studies in-vivo: a model study with heptabarbital. J. Pharm. Pharmacol. 40: 552-557, 1988.

Dingemanse, J., Hutson, P.H., Langemeijer, M.W.E., Curzon, G. and Danhof, M.: Application of serial sampling of cerebrospinal fluid in pharmacodynamic studies with a drug active in the CNS: Heptabarbital concentrations at onset and offset of loss of righting reflex in rats. Neuropharmacol. 27: 467-474, 1988a.

Fuseau, E. and Sheiner, L.B. : Simultaneous modeling of pharmacokinetics and

pharmacodynamics with a nonparametric pharmacodynamic model. Clin. Pharmacol. Ther. 35: 733-741, 1984.

Gregory, T.K. and Pettus, D.C.: An electroencephalographic processing algorithm specifically intended for analysis of cerebral activity. J. Clin. Monit. 2: 190-197, 1986.

Homer, T.D. and Stanski, D.R.: The effect of increasing age on thiopental disposition and anaesthetic requirement. Anaesthesiology 62: 714-724, 1985.

Mandema, J.W. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the central nervous system effects of heptabarbital using aperiodic EEG analysis. J. Pharmacokin. Biopharm. 18: 459-481, 1990.

Roberts, P., Turnbull, M.J. and Winterburn, A.: Diurnal variation in sensitivity to and metabolism of barbiturate in the rat: lack of correlation between *in vivo* and *in vitro* findings. Eur. J. Pharmacol. 12: 375-377, 1970.

Stanski, D.R. and Maitre P.O.: Population pharmacokinetics and pharmacodynamics of thiopental: The effect of age revisited. Anaesthesiology 72: 412-422, 1990.

Stijnen, A.M., Danhof, M. and Van Bezooijen, C.F.A.: Increased sensitivity to the anaesthetic effect of phenobarbital in ageing BN/BiRij rats. J. Pharmacol. Exp. Ther., in press.

Stijnen, A.M., Velders-van de Voort, S.H., Zurcher, C., Danhof, M. and Van Bezooijen, C.F.A.: The influence of concurrent disease on age-related changes in the pharmacokinetic-pharmacodynamic relationship of heptabarbital in rats. Submitted for publication.

Chapter 8

PHARMACOKINETIC-EEG EFFECT RELATIONSHIPS OF MIDAZOLAM IN AGEING BN/BIRIJ RATS

S. Hovinga[1,*], A.M. Stijnen[1,2], M.W.E. Langemeijer[1], J.W. Mandema[1], C.F.A. van Bezooijen[2] and M. Danhof[1]

*Deceased January 30, 1991

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, The Netherlands
[2]TNO Institute for Ageing and Vascular Research, Leiden, The Netherlands

submitted for publication

Summary

The purpose of the present investigations was to determine the influence of increasing age on the pharmacokinetics and pharmacodynamics of midazolam in male BN/BiRij rats as an animal model of ageing. Midazolam was administered intravenously at a dose of 2.5 mg/kg and its pharmacokinetics were determined on the basis of plasma concentrations as measured by HPLC. Pharmacodynamics were studied using the midazolam-induced changes in the electroencephalogram (EEG) as a measure of the pharmacological effect. Results were evaluated on the basis of simultaneous pharmacokinetic-pharmacodynamic modelling. In an attempt to differentiate between the effects of ageing on one hand and of concurrent disease on the other, an extensive clinical biochemical/pathological examination was conducted in individual rats by an independent pathologist.
The pharmacokinetics of midazolam were best characterized on the basis of

a two exponential model. In the 4-month-old rats the values of the clearance, volume of distribution and elimination half-life were 104 ± 13 ml/min.kg (mean ± SEM), 3.4 ± 0.7 l/kg and 30 ± 3 min, respectively. With increasing age, no changes in the pharmacokinetics of midazolam were observed.
The pharmacodynamics of midazolam were determined on the basis of the sigmoidal E_{max} model. In the 4-month-old rats the values of the parameters relative maximum effect, midazolam concentration at half maximum effect and Hill factor were 106 ± 10%, 50 ± 6 μg/l and 1.6 ± 0.3, respectively. In the group as a whole no significant changes in the pharmacodynamic parameters of midazolam were observed. However, when diseased animals were excluded from the evaluation, a tendency towards a decrease in the midazolam concentration at half maximum effect to 25 ± 14 μg/l was observed in the 36-month-old rats.
These findings suggest that increasing age is associated with a tendency towards an increased brain sensitivity to midazolam, which is reflected in a parallel shift of the concentration vs. EEG effect relationship towards lower concentrations. However, it appears that also factors other than age contribute to interindividual variability in pharmacodynamics as well, considering the substantial interindividual variability within certain age groups.

Introduction

During the last years, midazolam has become an important intravenous sedative, because of its favourable properties, e.g. short elimination half-life, water solubility and lack of important adverse effects (Dundee et al., 1984). Due to the cardiorespiratory stability after its administration, midazolam is especially preferable above other anaesthetics for induction of anaesthesia in poor-risk, elderly and cardiac patients (Dundee et al., 1984). Also in the treatment of sleep disorders in the elderly, midazolam was reported to be effective and well-tolerated, provided that it is given in a carefully titrated, appropriate dose (Lachnit et al., 1983). Several authors emphasized the importance of titrating the dose, since dose requirement for midazolam is reduced in elderly patients (Kanto et al., 1986; Oldenhof et al., 1988; Bell et al., 1987). In a study in 800 patients in which the dose of intravenous midazolam required to produce adequate sedation prior to upper gastro-intestinal endoscopy was measured, an important decrease of approximately 75% in dose requirement from the age of 15 years to the age of 85 years was

observed (Bell et al., 1987).
An important question is, whether the decrease in dose requirement is caused by pharmacokinetic and/or pharmacodynamic changes during ageing. The purpose of the present investigations was therefore to study the pharmacokinetics and the pharmacodynamics of midazolam simultaneously, using the midazolam-induced changes in the electroencephalogram (EEG) as a measure for the pharmacological effect. The experiments were performed in an animal model of ageing (BN/BiRij rats).

Materials and methods

Animals

Five groups of male BN/BiRij rats (TNO Institute for Ageing and Vascular Research, Leiden, The Netherlands) of different ages (4, 13, 24 and 29 and 36 months) were used. The 10, 50 and 90 % survival ages of this strain are 38.1, 31.7 and 22.8 months, respectively. Water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands) were supplied *ad libitum*. The rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature was maintained at 22-23 °C.

Chemicals

Midazolam was kindly donated by Hoffmann-La Roche, Basel, Switzerland.

Animal experiments

1. Clinical biochemical/pathological evaluation
In order to be able to determine the health status of the animals, several clinical biochemical indices were measured (blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase and glucose, total plasma protein and albumin concentration, urine production, urine osmolality and creatinine clearance). The concentrations of creatinine in blood

and urine were determined using a Reflotron autoanalyzer (Boehringer, Mannheim, Germany). The other indices were determined as described before (Stijnen et al., submitted; chapter 6).
After the pharmacokinetic and pharmacodynamic evaluation, the animals were sacrificed, the liver was weighed, and the heart, the lungs, the kidneys, the liver, the brains and macroscopically visible abnormalities were evaluated microscopically by a pathologist. Details about this evaluation were reported before (Stijnen et al., submitted; chapter 6).

2. Pharmacokinetic/pharmacodynamic evaluation
Cortical EEG electrodes were implanted two weeks before the experiment, using the method described by Mandema and Danhof (1990). Five hours before the experiments cannulas were implanted in the femoral vein and femoral artery under halothane anaesthesia. All experiments were started around 2.00 P.M. in order to minimize the influence of a possible diurnal rhythm in brain sensitivity, baseline EEG characteristics and/ or metabolism rate (Roberts et al., 1970). During the experiments the rats were kept in a slowly rotating drum to keep the vigilance of the animals constant.
After about 15 minutes baseline EEG recording, midazolam was administered as an intravenous dose of 2.5 mg/kg (dissolved in saline with an equimolar quantity of hydrochloric acid) in 5 minutes. Arterial blood samples of 100 µl (or 200 µl near the end of the experiment) were taken throughout the whole experiment. The EEG was continuously recorded during the first 60 minutes after drug administration (rotating drum on). After that a scheme of 4 minutes rotating drum off and 6 minutes on was followed in order not to fatigue the old animals. Only the EEG recorded during the last 5 minutes of the 6 minutes of the 'drum on' period was analyzed. The experiment was terminated after the EEG returned to a value within 10% of the baseline level.
By applying aperiodic analysis (Gregory and Pettus, 1986) the total amplitude in the frequency area of 11.5 to 30 Hz of the fronto-central EEG lead was calculated and used as the descriptor of the drug effect, because this was shown to provide an optimal measure for the effect of midazolam on the central nervous system (Bührer et al., 1990; Mandema et al., 1991). More detailed information on the recording and analysis of the EEG are described by Mandema et al. (1991).

Drug analysis

The concentrations of midazolam in plasma were measured by the HPLC-method and apparatus described by Mandema et al. (1991). Part of the samples were measured using a slightly modified method: instead of 25 ng diazepam 37.5 ng clobazam was used as the internal standard, 5mM octansulfonic acid was added to the eluent and the flow was reduced to 0.8 ml/min, yielding retention times of 3.8 and 6.5 minutes for clobazam and midazolam, respectively. Coefficients of variation were less than 5% and the detection limit was about 10 µg/l.
In order to determine the plasma protein binding in each individual animal, a second intravenous dose of 2.5 mg/kg was administered to the rat about three days after the experiment and a blood sample of 2 ml was withdrawn via a cut in the tail at 1 minute following administration. The blood sample was subjected to ultrafiltration and the unbound concentration of midazolam in the ultrafiltrate was measured in the same way as the plasma samples.

Data analysis

1. Pharmacokinetic-pharmacodynamic modelling
The midazolam plasma concentration vs. time profile was described using a two exponential equation for intravenous infusion. The total body clearance, the steady state volume of distribution, the volume of the central compartment and the elimination half-life were calculated from the coefficients and exponents of the fitted functions according to standard procedures (Gibaldi and Perrier, 1982).
The relationship between the concentrations (calculated at several time points using the pharmacokinetic model) and the increase in the total amplitude in the frequency range of 11.5-30 Hz (as the measure for the EEG-effect) was characterized by the sigmoidal E_{max} model (Mandema et al., 1991). In 44% of the animals an overshoot in the effect time profile immediately after drug administration was observed, which lasted longer than 5 minutes. The early peak effects were not related to the midazolam concentration. These early peak effects were omitted in the characterization by the sigmoidal E_{max} model. This was considered justified, while the concentration vs. EEG effect relationship of the animals showing early peak effects were not different from those not showing these effects.

All calculations were performed using the nonlinear least squares regression program Siphar (Simed SA, Creteil, France).

2. Statistics

The effect of ageing on the biochemical indices, the pharmacokinetic and the pharmacodynamic parameters was statistically tested by one-way analysis of variance and the Student's t-test with Bonferroni correction. In case of nonhomogeneity of variances (assessed by Bartlett's test) the Welch test and the multiple Welch test were used. P-values lower than 0.05 were judged to be significant.

Results

1. Clinical biochemical/pathological evaluation

The results of the clinical biochemical indices are shown in table 1 for all animals. An age-related decrease was measured for osmolality and blood alanine aminotransferase, a decrease followed by an increase for blood urea nitrogen and the ratio liver weight/ body weight and a tendency to decrease for the creatinine clearance.

The pathological evaluation resulted in five animals suffering from diseases, which were excluded from the evaluation of the results of the study. One 13-month-old animal displayed meningeal periarteritis, one 29-month-old a necrotizing urothelial tumour of renal pelvis, two 36-month-old rats showed several lesions in the liver (necrosis, focal inflammation and cysts in both, with abscesses in one of the two rats and severe bile duct proliferation in the other one) and another 36-month-old rat showed endocardial schwannoma of the heart, signs of cardiac failure and an adrenocortical carcinoma.

2. Pharmacokinetic/pharmacodynamic evaluation

Figure 1 shows the plasma concentration vs. time profile and the EEG effect vs. time profile in one typical rat.

Since no time delay was observed between plasma concentration and EEG effect (in any age group), these two were directly correlated to each other and characterized by the sigmoidal E_{max} model (figure 2). Figures 3 and 4 display the estimates of all individual animals (including the diseased ones) for the pharmacokinetic and pharmacodynamic parameters for the different age groups, respectively. The mean values in the figures and in tables 2 and 3

Table 1. *Effect of age on selected clinical biochemical indices.*

age (month)	4	13	24	29	36
number of animals	8	9	8	8	6
body weight (g)	224 ± 10	398 ± 12[a]	398 ± 11[a]	405 ± 11[a]	388 ± 13[a]
liver weight (g)	7.7 ± 0.4 (n=6)	9.6 ± 0.7 (n=8)	9.6 ± 0.4 (n=5)	9.7 ± 0.5	9.5 ± 1.4
liver wt/ body wt (%)	3.68 ± 0.16 (n=6)	2.36 ± 0.11[a] (n=8)	2.43 ± 0.22[a] (n=5)	2.40 ± 0.11[a]	2.92 ± 0.26
blood aspartate aminotransferase (I.U./l)	102.3 ± 7.1	94.2 ± 6.9	76.9 ± 5.6	82.7 ± 3.4	105 ± 11
blood alanine aminotransferase (I.U./l)	44.8 ± 2.5	40.8 ± 2.1	34.0 ± 1.7[a]	35.2 ± 1.5	29.6 ± 3.3[a]
plasma albumin (mg/ml)	35.9 ± 1.8	32.2 ± 1.9	29.4 ± 1.8	33.0 ± 1.3	36.3 ± 2.5
plasma total protein (mg/ml)	81.5 ± 2.8	88.6 ± 3.2	92.1 ± 1.6	94.6 ± 1.3[a]	93.2 ± 4.7
blood glucose (mmol/l)	4.7 ± 0.3	4.5 ± 1.8	4.4 ± 0.3	4.2 ± 0.2	3.9 ± 0.5
blood urea nitrogen (mmol/l)	8.3 ± 0.4	7.4 ± 0.2	6.0 ± 0.4[a,b]	6.5 ± 0.4[a]	8.2 ± 0.9
creatinine clearance (ml/h.kg)	174 ± 26	151 ± 18	157 ± 14	142 ± 14	94 ± 19
urine production (ml/24 hrs)	8.3 ± 0.6	7.7 ± 1.1	9.3 ± 1.6	9.4 ± 1.3	11.4 ± 1.3
osmolality (mOsm/l)	1980 ± 140	1550 ± 140	1340 ± 91[a]	1174 ± 71[a]	1135 ± 75[a]

Results are presented as mean ± SEM

[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 13-month value, $p < 0.05$

were calculated including only the healthy animals.

Total body clearance, steady state volume of distribution, volume of the central compartment and elimination half-life did not show significant changes during ageing (table 2 and figure 3). Also plasma protein binding and the fraction unbound did not change during ageing (fraction unbound: 4.6 ± 0.9%, 4.6 ± 0.6%, 4.6 ± 0.7%, 3.9 ± 0.3% and 4.3 ± 0.9% (mean ± SEM) for the 4, 13, 24, 29 and 36-month-old rats, respectively).

Concerning the pharmacodynamics (table 3 and figure 4), the EEG baseline effect, the absolute and relative maximum effect and the Hill factor did not change during ageing. The total and free (unbound) midazolam concentration at half the maximum effect showed a tendency to decrease between the ages of 29 and 36 months. This tendency was only found when the diseased animals were excluded.

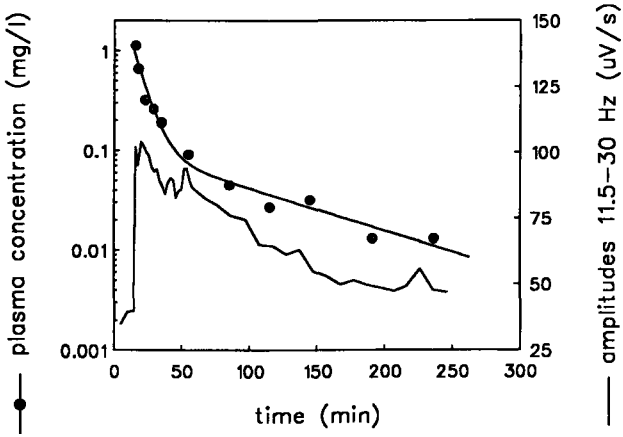

Figure 1. Midazolam plasma concentration vs. time profile (the solid line through the data points represents the characterization by a two exponential equation) and EEG effect vs. time profile for a typical rat (4 months of age) using the total amplitude in the frequency range of 11.5-30 Hz as the descriptor of the EEG effect. The first 15 minutes in the effect vs. time profile represent the baseline recording. Because the 5 minutes infusion time is left out in this figure, time point 15 minutes is the end of the midazolam administration.

Table 2. *The influence of ageing on pharmacokinetic parameters of midazolam following an intravenous dose of 2.5 mg/kg (considering only healthy animals).*

age (month)	4	13	24	29	36
number of animals	8	8	8	7	3
total body clearance (ml/min.kg)	104 ± 13	96 ± 11	75 ± 11	66 ± 9	78 ± 11
steady state volume of distribution (l/kg)	3.4 ± 0.7	2.9 ± 0.2	3.6 ± 0.5	3.3 ± 0.5	3.6 ± 1.3
volume of the central compartment (l/kg)	1.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	1.1 ± 0.2	1.5 ± 0.3
elimination half-life (min)	30 ± 3	32 ± 4	49 ± 2	50 ± 6	43 ± 14

The results are presented as mean ± SEM

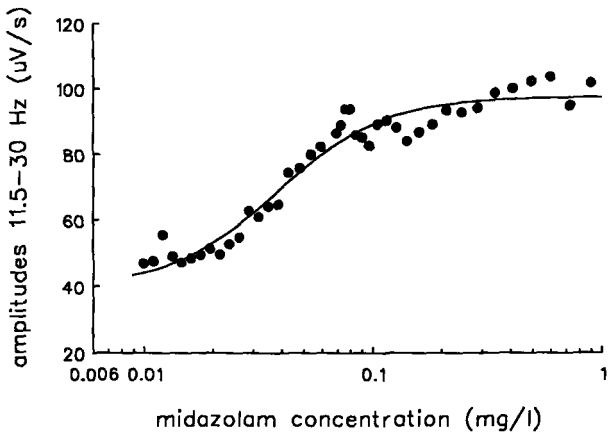

Figure 2. Plasma concentration vs. EEG effect relationship for a typical 4-month-old rat. The solid line through the data points represents the characterization of the concentration vs. effect relationship by the sigmoidal E_{max} model.

Discussion

A few years ago, Bell et al. (1987) reported a remarkable age-related decrease in dose requirement of intravenous midazolam to produce adequate sedation to perform upper gastro-intestinal endoscopy. In a clinical study in about 800 patients a reduction in dose requirement of 75 % between the ages of 15 and 85 years was observed. The mechanism of the decrease in dose requirement was not studied; this can be relatively complex with a multitude of pharmacokinetic and pharmacodynamic factors involved. With regard to the pharmacokinetics, especially changes in the (central) volume of distribution are an important determinant (Greenblatt et al., 1984); for the pharmacodynamics changes in both E_{max} and EC_{50} can be relevant.

In a number of investigations, the influence of increasing age on the pharmacokinetics and pharmacodynamics of benzodiazepines has been studied. This has resulted in a situation, where the influence of increasing age on the pharmacokinetics of various benzodiazepines has been rather well documented (Danhof, 1991). The situation with regard to the pharmacodynamics is, however, much less clear. In many instances, the

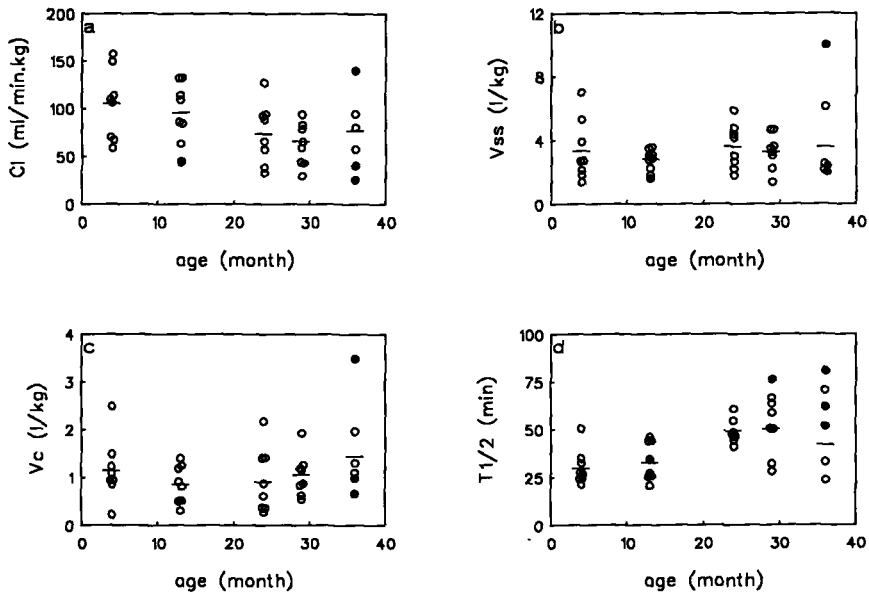

Figure 3. Individual values (of both diseased and healthy animals) and mean value (of only the healthy animals) of the pharmacokinetic parameters in the different age groups following intravenous administration of midazolam at a dose of 2.5 mg/kg.
o healthy animals; ● diseased animals; - mean value of the healthy animals

a. total body clearance (Cl)
b. steady state volume of distribution (Vss)
c. volume of the central compartment (Vc)
d. elimination half-life (T1/2)

effects on pharmacodynamics have been characterized on the basis of psychomotor performance tests. The results of these investigations have been rather inconclusive. Complicating factors have been the occurrence of age-related changes in the baseline levels of the performance measures and the interference of various complicating pharmacokinetic factors (i.e. the role of active metabolites and changes in distribution), of which the exact contribution has often not been determined. A limitation of studies in humans is that further mechanistic studies are not possible.

In the present investigation, we studied the influence of increasing age on the pharmacokinetics and pharmacodynamics of midazolam in BN/BiRij rats as an animal model of ageing. The survival curves and the age-related pathology

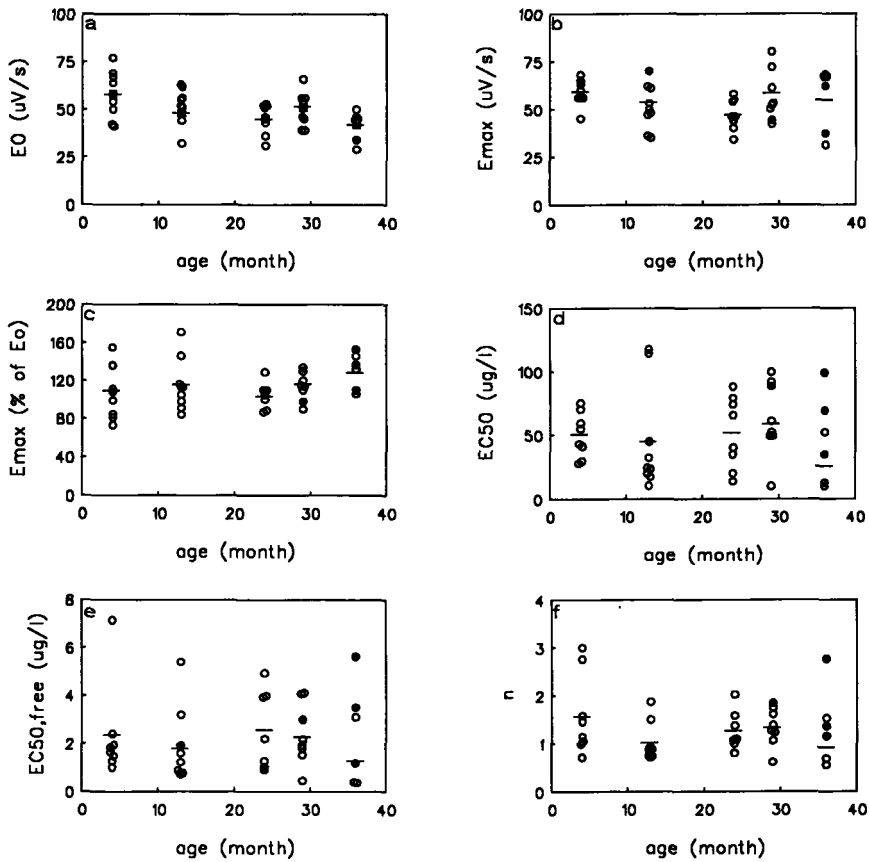

Figure 4. Individual values (of both diseased and healthy animals) and mean value (of only the healthy animals) of the sigmoidal E_{max} parameters in the different age groups following intravenous administration of midazolam at a dose of 2.5 mg/kg.
o healthy animals; ● diseased animals; - mean value of the healthy animals

a. baseline effect value (E0)
b. absolute maximum effect (Emax)
c. relative maximum effect (Emax expressed as percentage of E0)
d. plasma concentration at half the maximum effect (EC50)
e. unbound plasma concentration at half the maximum effect ($EC50_{,free}$)
f. shape factor (n)

of this rat strain are well characterized and therefore this rat strain is suitable for this type of investigations (Burek, 1978; Hollander, 1979). The increase in the amplitude in the 11.5-30 Hz frequency range of the EEG was used as a

Table 3. *The influence of ageing on the concentration vs. EEG-effect relationship parameters of midazolam (considering only healthy animals).*

age (month)	4	13	24	29	36
number of animals	8	8	8	7	3
baseline effect value (μV/s)	58 ± 5	48 ± 4	45 ± 3	51 ± 4	42 ± 7
absolute maximum effect (μV/s)	59 ± 3	54 ± 4	47 ± 3	59 ± 5	55 ± 12
relative maximum effect (% of baseline effect)	106 ± 10	116 ± 10	105 ± 5	116 ± 6	129 ± 12
plasma concentration at half the maximum effect (μg/l)	50 ± 6	45 ± 16	52 ± 10	59 ± 11	25 ± 14
unbound plasma concentration at half the maximum effect (μg/l)	2.4 ± 0.7	1.8 ± 0.6	2.6 ± 0.6	2.3 ± 0.5	1.3 ± 0.9
shape factor	1.6 ± 0.3	1.0 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	0.9 ± 0.3

The results are presented as mean ± SEM

pharmacodynamic measure. This parameter is easily measured in rats (Mandema et al., 1991) and has also been found pharmacologically relevant in that it reflects the effect of benzodiazepines in GABA-ergic neurotransmission (Mandema et al., 1991a). It was demonstrated that (inter)active metabolites do not interfere with the pharmacological response of midazolam and that (acute) functional tolerance development does not occur (Mandema et al., 1991; Mandema J.W., Hoogerkamp, A. and Danhof, M.; unpublished observations).

The values of the pharmacokinetic parameters of midazolam in the BN/BiRij rats of the present study were found to be slightly different from those observed previously in male Wistar rats. For both the systemic clearance and the volume of distribution larger values were observed in BN/BiRij rats relative to Wistar rats. The values of the elimination half-life were similar (Mandema et al., 1991, 1991a). With increasing age no changes in the values of the various pharmacokinetic parameters of midazolam were observed. For both central and steady state volumes of distribution, this appears to be in line with observations in humans (Greenblatt et al., 1984; Smith et al., 1984). This implies that changes in the volume parameters cannot explain the decreased dose requirement reported by Bell et al. (1987). In humans, a lower total body clearance and an increased elimination half-life were found in elderly males, but not in females (Greenblatt et al., 1984; Servin et al., 1987). The difference between the findings in humans and those in rats might be explained by a

comparable difference in the influence of age on liver weight, which decreases during ageing in man (Thompson and Williams, 1965) and does not change in our rats (table 1). In the present study, a wide interindividual variability in pharmacokinetic parameter estimates was observed, as was also reported in humans (Servin et al., 1987). No clear relationship with the presence of concurrent disease could be detected.

The values of the pharmacodynamic parameter estimates of midazolam in the BN/BiRij rats in the present investigation were also slightly different from those obtained in Wistar rats. The values of the maximum effect and the concentration at half the maximum effect were lower and that of the shape factor was higher than the values in Wistar rats, whereas the baseline effect values were comparable (Mandema et al., 1991a).
In the group as a whole the pharmacodynamic parameter estimates in the BN/BiRij rats showed significant interindividual variability, but no clear age-related changes in pharmacodynamics were observed. In humans, also a high variation in pharmacological response was reported in young adults (Dundee et al., 1984). In previous investigations it has been demonstrated however that the results of pharmacodynamic investigations with drugs acting on the central nervous system can be confounded by the presence of concurrent disease (Stijnen et al., submitted; chapter 6). Therefore, an extensive pathological evaluation was performed by an experienced research pathologist in the animals of this study. This resulted in one 13-month-old animal, one 29-month-old animal and three out of the total of six animals in the 36-month-old group being diseased. When these animals were excluded from the evaluation, on average a decrease in the concentration at half the maximum effect from 50 ± 6 μg/l (mean ± SEM) in the 4-month-old rats to 25 ± 14 μg/l in the 36-month-old rats was observed, suggesting indeed an increased brain sensitivity to the pharmacological effects of midazolam with increasing age. Due to the limited number of healthy 36-month-old animals however, this difference did not reach statistical significance.

Baughman et al. (1987), upon studying the EEG effect of midazolam qualitatively, did not find differences between young (6-month-old) and old (28-month-old) rats. However, they measured an age-related increased effect of the drug on the cerebral blood flow (CBF) and the cerebral oxygen consumption ($CMRO_2$). These effects were reported to decrease coincident

with the sedative/hypnotic effect (Hoffman et al., 1986). Baughman et al. stated that a depression of non-electric metabolic function by midazolam in the aged rat would be important in this respect.

Many investigators have examined the influence of ageing on the pharmacodynamics of benzodiazepines generally on the basis of investigations in vitro. Emphasis in these studies has been on distinct aspects like for example the binding of benzodiazepines and gamma-aminobutyric acid (GABA) to the GABA-benzodiazepine receptor complex as well as functioning of the chloride channel. The results of these investigations have been rather inconclusive, however (Pedigo et al., 1981; Reeves and Schweizer, 1983; Kochman and Sepulveda, 1986; Concas et al., 1988; Ito et al., 1988). A relative disadvantage of these investigations in vitro is that only a single aspect of the pharmacodynamics is studied. In studies *in vivo*, the entire pharmacodynamic process is studied including binding to relevant receptors, processes that occur following binding to the receptor as well as *in vivo* homeostatic processes that may be operative. Thus on the basis of the *in vivo* investigations, an impression of changes in the overall pharmacodynamic process is obtained.

In conclusion, the findings of the present investigation show that there is a significant interindividual variability in the pharmacodynamics of midazolam in rats. Increasing age *per se* however, appears to be only a minor factor causing this variability. In this respect, a large investigation is currently in progress on data obtained in some 150 rats where population analysis is used to identify factors that may be important in this respect. Similar studies are indicated in man and it is of interest that the utilized EEG parameter can also be applied in human studies (Mandema et al., submitted).

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References

Bell, G.D., Spickett, G.P., Reeve, P.A., Morden A. and Logan, R.F.A.: Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. Br. J. clin. Pharmacol. 23: 241-244, 1987.

Baughman, V.L., Hoffman, W.E., Albrecht, R.F. and Miletech, D.J.: Cerebral vascular and metabolic effects of fentanyl and midazolam in young and aged rats. Anesthesiol. 67: 314-319, 1987.

Bührer, M., Maitre, P.O., Hung, O. and Stanski, D.R.: Electroencephalographic effects of benzodiazepines. I. Choosing an electroencephalographic parameter to measure the effect of midazolam on the central nervous system. Clin. Pharmacol. Ther. 48: 544-554, 1990.

Burek, J.D.: Pathology of ageing rats. A morphological and experimental study of age-associated lesions in aging BN/Bi, Wag/Rij and (Wag x BN) F_1 rats. CRC-Press inc., West Palm Beach, Florida, 1978.

Concas, A., Pepitoni, S.J., Atsoggiu, T., Toffano, G. and Biggio, G.: Aging reduces the GABA dependent ^{36}Clflux in rat brain membrane vesicles. Life Sci. 43: 1761-1771, 1988.

Danhof, M.: Pharmacology of hypnotics in the elderly. in: Sleep and ageing. S. Smirne, M. Franceschi, L. Ferini-Strambi, eds., Masson, Milano, 1991, pp 217-224.

Dundee, J.W., Halliday, N.J., Harper, K.W. and Brogden, R.N.: Midazolam. A review of its pharmacological properties and therapeutic use. Drugs 28: 519-543, 1984.

Gibaldi, M. and Perrier, D.: Noncompartmental analysis based on statistical moment theory. In: Pharmacokinetics. 2nd ed., Marcel Dekker, New York, 1982, pp 409-424.

Gregory, T.K. and Pettus, D.C.: An electroencephalographic processing algorithm specifically intended for analysis of cerebral activity. J. Clin. Monit. 2: 190-197, 1986.

Greenblatt, D.J., Abernethy, D.R., Lockniskar, A., Harmatz, J.S., Limjuco, R.A. and Shader, R.I.: Effect of age, gender and obesity on midazolam kinetics. Anesthesiol. 61: 27-35, 1984.

Hoffman, W.E., Miletich, D.J. and Albrecht, R.F.: The effects of midazolam on cerebral blood flow and oxygen consumption and its interaction with nitrous oxide. Anesth. Analg. 65: 729-733, 1986.

Hollander, C.F.: Pathophysiology of ageing in animal models. in: Drugs and the elderly. Perspectives in geriatric clinical pharmacology. J. Crooks and I.H. Stevenson, eds., The MacMillan Press Ltd., 1979, pp 15-22.

Ito, Y., Ho, I.K. and Hoskins, B.: Cerebellar GABA and benzodiazepine receptor characteristics in young and aged mice. Brain Res. Bull. 21: 251-255, 1988.

Kanto, J., Aaltonen, L., Himberg, J-J. and Hovi-Viander, M.: Midazolam as an intravenous induction agent in the elderly: clinical and pharmacokinetic study. Anesth. Analg. 65: 15-20, 1986.

Kochman, R.L. and Sepulveda, C.K.: Aging does not alter the sensitivity of benzodazepine receptors to GABA modulation. Neurobiol. Aging 7: 363-365, 1986.

Lachnit, K.S., Proszowski, E. and Rieder, L.: Midazolam in the treatment of sleep disorders in geriatric patients. Br. J. clin. Pharmacol. 16, 173S-177S, 1983.

Mandema, J.W. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the central nervous system effects of heptabarbital using aperiodic EEG analysis. J. Pharmacokin.

Biopharm. 18: 459-481, 1990.

Mandema, J.W., Tukker, E. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of EEG effects of midazolam in individual rats: influence of rate and route of administration. Br. J. Pharmacol. 102: 663-668, 1991.

Mandema, J.W., Sansom, L.N., Dios-Viéitez, M.C., Hollander-Jansen, M. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the EEG effects of benzodiazepines. Correlation with receptor binding and anticonvulsant activity. J. Pharmacol. Exp. Ther. 257: 472-478, 1991a.

Mandema, J.W., Tuk, B., van Steveninck, Breimer, D.D., Cohen, A. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the CNS effects of midazolam and its main metabolite α-hydroxy-midazolam in healthy volunteers. submitted for publication.

Oldenhof, H., de Jong, M., Steenhoek, A. and Janknegt, R.: Clinical pharmacokinetics of midazolam in intensive care patients, a wide interpatient variability? Clin. Pharmacol. Ther. 43: 263-269, 1988.

Pedigo, N.W., Schoemaker, H., Morelli, M., McDougal, J.N., Malick, J.B., Burks, T.F. and Yamamura, H.I.: Benzodiazepine receptor binding in young, mature and senescent rat brain and kidney. Neurobiol. Aging 2: 83-88, 1981.

Reeves, P.M. and Schweizer, M.P.: Aging, diazepam exposure and benzodiazepine receptors in rat cortex. Brain Res. 70: 376-379, 1983.

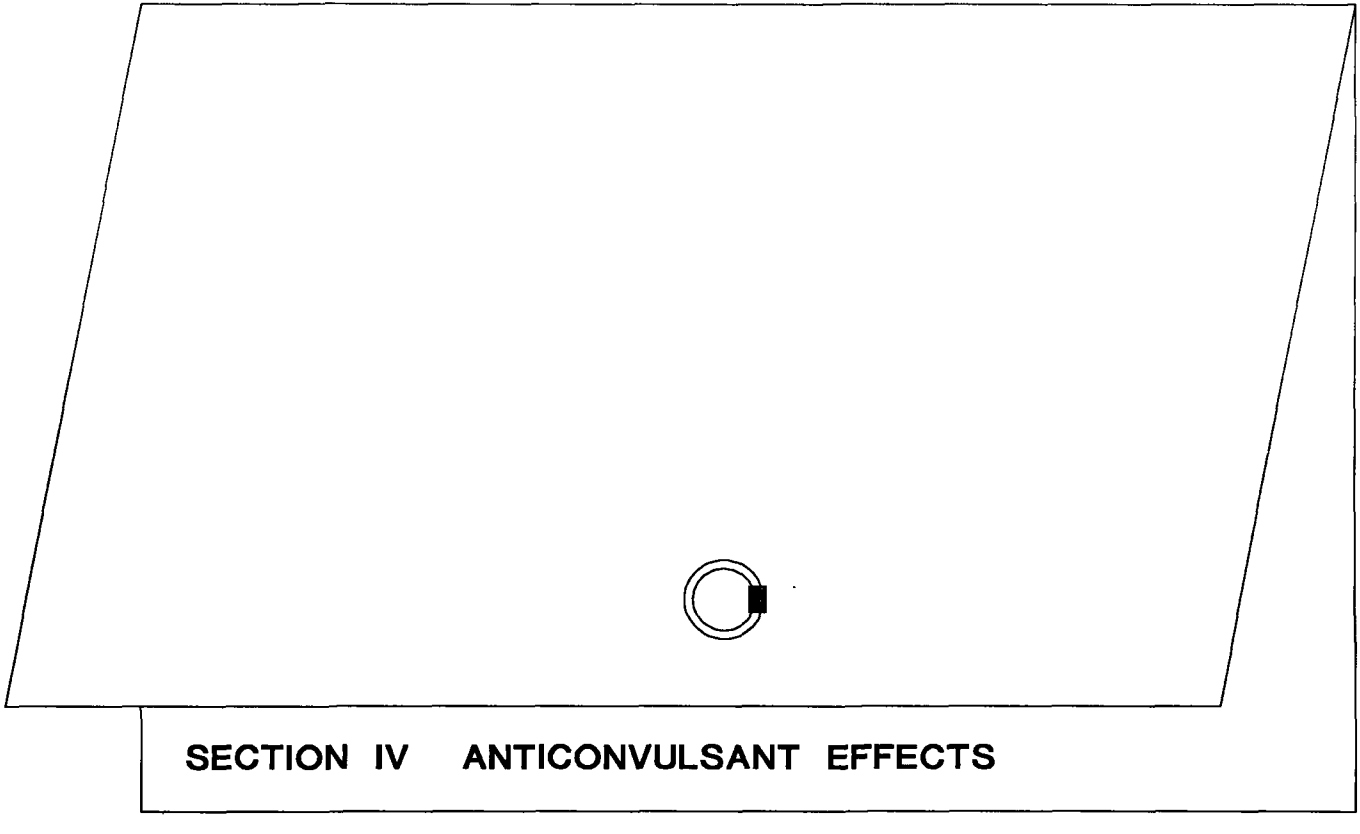
Roberts, P., Turnbull, M.J. and Winterburn, A.: Diurnal variation in sensitivity to and metabolism of barbiturate in the rat: lack of correlation between *in vivo* and in vitro findings. Eur. J. Pharmacol. 12: 375-377, 1970.

Servin, F., Enriquez, I., Fournet, M., Failler, J.M., Farinotti, R. and Desmonts, J-M.: Pharmacokinetics of midazolam used as an intravenous induction agent for patients over 80 years of age. Eur. J. Anaesth. 4: 1-7, 1987.

Smith, M.T., Heazlewood, V., Eadie, M.J., Brophy, T.O'R. and Tyrer, J.M.: Pharmacokinetics of midazolam in the aged. Eur. J. Clin. Pharmacol. 26: 381-388, 1984.

Stijnen, A.M., Velders-van der Voort, S.H., Zurcher, C., Danhof, M. and Van Bezooijen, C.F.A.: The influence of concurrent disease on age-related changes in the pharmacokinetic-pharmacodynamic relationship of heptabarbital in rats. Submitted for publication.

Thompson, E.N. and Williams, R.: Effect of age on liver function with particular reference to bromsulphalein excretion. Gut 6: 266-291, 1965.



SECTION IV ANTICONVULSANT EFFECTS

Chapter 9

PHARMACODYNAMICS OF THE ANTICONVULSANT EFFECT OF OXAZEPAM IN BN/BIRIJ RATS: EFFECTS OF A DIMINISHED HOMEOSTATIC RESERVE CAPACITY WITH INCREASING AGE

A.M. Stijnen[1,2], I. Postel-Westra[3,4], M.W.E. Langemeijer[1], A. Hoogerkamp[1], R.A. Voskuyl[3,4], C.F.A. van Bezooijen[2] and M. Danhof[1]

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, The Netherlands
[2]TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands
[3]Department of Physiology, University of Leiden, Leiden, The Netherlands
[4]Instituut voor Epilepsiebestrijding, Heemstede, The Netherlands

submitted for publication (slightly modified)

Summary

The purpose of this investigation was to examine the influence of increasing age on the pharmacokinetics and the time course of the anticonvulsant response of oxazepam in BN/BiRij rats as an animal model of ageing. Oxazepam was administered intravenously in a dose of 12 mg/kg body weight and the anticonvulsant effect intensity was measured as elevation above baseline of a threshold for induction of localized seizure activity (TLS) upon direct cortical stimulation with ramp shaped electrical pulse trains of increasing intensity.

No age-related changes in the pharmacokinetic parameters of oxazepam were observed. The pharmacological effect vs. time profile showed in young

rats an anticonvulsant component followed by proconvulsant component which is suggestive for the occurrence of acute tolerance and/or withdrawal syndrome. With increasing age the proconvulsant component disappeared, resulting in a monophasic effect profile (anticonvulsant effect only) at the age of 35 months with significantly higher anticonvulsant effect intensity immediately following drug administration.
In five animals of each age group benzodiazepine receptor binding characteristics were determined in vitro using [^{3}H]flunitrazepam as a ligand. Both receptor density and affinity did not show age-related changes. Available literature data on post-receptor events do not indicate conclusive age-related changes.
It is concluded that the observed change in the pharmacodynamics of the anticonvulsant effect of oxazepam can be explained by the disappearance of the tolerance/withdrawal phenomenon. This is compatible with a decreased efficiency of homeostatic control mechanisms in the elderly.

Introduction

It has been frequently reported that with increasing age there is an increase of the sensitivity of the brain to the pharmacological actions of benzodiazepines, especially for sedative effects and the effects on psychomotor function (Castleden et al., 1977; Reidenberg et al., 1978; Kanto et al., 1981; Swift et al., 1981, 1985, 1985a; Cook, 1986). Thus far, the influence of increasing age on the anticonvulsant effect of benzodiazepines has not been studied extensively. This may be relevant, because with increasing age there is an increase in the incidence of epilepsy and in the use of antiepileptic drugs (Hauser and Kurland, 1975; Troupin and Johannessen, 1990). Moreover, the mechanism of the anticonvulsant effect of benzodiazepines may be in part different from that of most of the other effects of these drugs, because of the involvement of micromolar affinity benzodiazepine receptors (Bowling and De Lorenzo, 1982).
The purpose of this investigation was to study the influence of increasing age on the pharmacodynamics of the anticonvulsant effect of oxazepam *in vivo*. Since changes in pharmacodynamics may occur simultaneously at different organizational levels (i.e. receptor binding characteristics, post-receptor events or homeostatic mechanisms), it was chosen to study the pharmacodynamics initially *in vivo*, i.e. in the complete living system.

Anticonvulsant effect intensity was determined on the basis of the threshold for induction of a localized seizure reaction upon direct cortical stimulation with ramp shaped electrical pulse trains of increasing intensity (Voskuyl et al., 1989). In combination with the determination of blood concentrations and the application of simultaneous pharmacokinetic-pharmacodynamic modelling concepts, this allows the determination of concentration vs. anticonvulsant effect relationships in individual rats (Dingemanse et al., 1990). In addition, benzodiazepine receptor binding characteristics were determined in vitro using [^{3}H]flunitrazepam as radioligand.

Materials and methods

Animals

Five groups of male BN/BiRij rats (TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands) of different ages (4, 12, 24, 31 and 35 months; n = 8, 8, 7, 6, 10, respectively) were used. The 10, 50 and 90 % survival ages of the male BN/BiRij rats are 38.1, 31.7 and 22.8 months, respectively. During the period in which the experiments were performed, the rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature was maintained at 22-23 °C. They were allowed free access to water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands).

Chemicals

Oxazepam was a generous gift from Wyeth BV (Hoofddorp, The Netherlands) and flunitrazepam from Hoffmann - La Roche (Mijdrecht, The Netherlands). [^{3}H]-flunitrazepam (specific activity 87 Ci/mmol) was obtained from New England Nuclear ('s-Hertogenbosch, The Netherlands) and N,N-Dimethylacetamide (DMA) from Merck Schuchardt (München, Germany).

Animal experiments

1. Clinical biochemical/pathological evaluation

In order to be able to determine the health status of the animals, several clinical biochemical parameters were measured. Blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase, glucose, total plasma protein and albumin concentration, 24 hour urine production, osmolality, and creatinine clearance were determined as described before (Stijnen et al., submitted; chapter 6).

After the pharmacokinetic/pharmacodynamic evaluation, the animals were sacrificed, the liver was weighed and the heart, the lungs, the kidneys, the liver, the brain and macroscopically visible abnormalities were evaluated by a pathologist, who was unaware of the outcome of the pharmacokinetic/pharmacodynamic evaluation.

2. Pharmacokinetic/pharmacodynamic evaluation

Three weeks before the experiment two electrodes were implanted in the skull over the motor area of the frontoparietal cortex as described by Voskuyl et al. (1989). A bipolar pulse train (duration of one pulse 2 ms, frequency 50 pulses per s) of linearly increasing amplitude (0-2400 μA in 20 s), applied to the electrodes, was used as the convulsive stimulus and the threshold to induce localized seizure activity was determined, by measuring the stimulus current corresponding to the (visually determined) point at which clonic forelimb activity started. Since the convulsive threshold, which would be measured repeatedly in the experiment, decreases in the initial test sessions (Voskuyl et al., 1989), this threshold was determined twice daily during the two weeks before the actual experiment until a stable baseline threshold was reached. One day before the experiment, a polyethylene cannula was implanted in the right jugular vein under light halothane anaesthesia. On the day of the experiment the threshold to induce localized seizure activity was determined five times before the administration of oxazepam; the mean threshold value was used as the baseline level. Anticonvulsant effect intensities after administration of oxazepam were expressed as the elevation in threshold over this baseline level.

2a. In vivo experiment

Oxazepam, dissolved in 100 µl dimethylacetamide, was administered via the cannula in the jugular vein as an intravenous bolus dose of 12 mg/kg in about one minute to the rats of the five different age groups. The animals of a 4-month-old control group only received the solvent. The seizure threshold was determined repeatedly (38 times) and 16 blood samples of 100µl were collected from an incision in the tail during a period of 7 hours after administration (in 5 of the 35-month-old rats the experiment was extended to a total of 11 hours). Blood samples were immediately haemolyzed by addition of 500 µl of distilled water and stored at -20°C until analysis. In order to determine *in vivo* plasma protein binding, a second oxazepam bolus dose (6 mg/kg) was administered to the rats one day after the experiment and one minute thereafter a blood sample of about 2 ml was withdrawn from an incision in the tail. Plasma was separated from the blood samples and stored at -20°C until analysis. A few days later the brain was removed in order to determine the in vitro benzodiazepine receptor binding characteristics. The brains were stored at -70°C until analysis.

2b. Receptor binding

The in vitro benzodiazepine receptor binding characteristics were determined at 0°C using [^{3}H]flunitrazepam as a ligand applying the method described by Hollander-Jansen et al. (1989).

Drug analysis

Whole blood oxazepam concentrations were measured by an HPLC-method described by Dingemanse et al. (1988) with slight modifications (oxazepam concentrations were measured in 100 µl whole blood in stead of in 100 µl plasma and the 100 µl samples were diluted with distilled water instead of with borate buffer). The HPLC-system (Kratos Analytical Instruments, Ramsey, USA) consisted of a Spectroflow 400 solvent delivery system, a Promis automatic sample injector and a Spectroflow 757 UV detector, set at 240 nm. A Z-module containing a Radial-Pak C-18 cartridge, particle size 10 µm, was used (Waters Ass., Milford, USA). For data processing a Shimadzu C-R3A reporting integrator was applied. Protein binding of oxazepam was determined by means of ultrafiltration using the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands). The concentrations of oxazepam in the

ultrafiltrate were measured in the same way as the blood samples.

Data analysis

1. Pharmacokinetics

The area under the oxazepam concentration-time curve (AUC) was calculated using the linear trapezoidal rule with extrapolation to infinity, using the elimination rate constant k. This elimination rate constant was determined using the slope of the terminal phase of the log concentration vs. time profile. The elimination half life was calculated as 0.693/k. Total body clearance was calculated as dose/AUC and the apparent volume of distribution (V_{area}) as dose/AUC*k.

2. In vitro benzodiazepine receptor binding characteristics

The receptor binding data were subjected to a Langmuir-type equation (Hollander-Jansen et al., 1989):

$$\text{number of receptors occupied} = \frac{B_{max} \cdot C}{K_D + C}$$

in which B_{max} represents the total number of specific binding sites, K_D the apparent dissociation constant and C the free flunitrazepam concentration, applying a least squares nonlinear regression algorithm (Siphar, Simed SA, Creteil, France).

3. Statistics

Differences between age groups were statistically evaluated by one-way analysis of variance followed by the Student's t-test with Bonferroni correction. Bartlett's test was used to assess homogeneity of variances. In case of nonhomogeneity of variances the Welch test and the multiple Welch test were used. P-values lower than 0.05 were judged to be significant.

Results

1. Clinical biochemical/ pathological evaluation

The results of the clinical biochemical indices are shown in table 1. The creatinine clearance and the osmolality decreased during ageing. In the other parameters very few changes were found in both the mean and the individual values, except for the values for blood aspartate and blood alanine aminotransferase in the 35-month-old animals, where the high SEM is caused by the extremely high individual values in one animal.

Three 35-month-old animals appeared to show pathological abnormalities. Two animals suffered from large meningeal granular cell tumours, in one of

Table 1. *Effect of age on selected clinical biochemical indices.*

age (month)	4	12	24	31	35
number of animals	8	8	7	6	10
body weight (g)	243 ± 10	397 ± 21[a]	377 ± 8[a]	429 ± 14[a]	376 ± 12[a]
liver weight (g)	8.6 ± 0.7	10.5 ± 0.6	10.9 ± 0.6 (n=6)	14.1 ± 0.7[a,b]	13.0 ± 1.0[a]
liver wt/ body wt (%)	3.49 ± 0.18	2.63 ± 0.06[a]	2.90 ± 0.12 (n=6)	3.29 ± 0.16	3.46 ± 0.25
blood aspartate aminotransferase (I.U./l)	101.5 ± 9.7	89.9 ± 5.5	81.5 ± 4.7	81.5 ± 4.1	163 ± 79
blood alanine aminotransferase (I.U./l)	38.4 ± 1.0	39.5 ± 1.0	36.7 ± 2.1	38.2 ± 4.9	68 ± 38
plasma albumin (mg/ml)	33.4 ± 0.6	32.8 ± 0.8	33.2 ± 0.9	35.2 ± 1.5	28.6 ± 2.8
plasma total protein (mg/ml)	82.8 ± 1.1	87.7 ± 1.5	88.4 ± 3.4	90.3 ± 4.7	80.9 ± 2.0
blood glucose (mmol/l)	5.0 ± 0.4	5.5 ± 0.2	5.4 ± 0.3	4.9 ± 0.4	4.8 ± 0.3
blood urea nitrogen (mmol/l)	9.5 ± 0.2	9.9 ± 0.6	8.4 ± 0.3[a]	8.5 ± 0.6	8.2 ± 0.2[a]
creatinine clearance (ml/h.kg)	297 ± 18	215 ± 11[a]	234 ± 22	158 ± 23[a]	152 ± 9[a,b,c]
urine production (ml/24 hrs)	8.6 ± 0.5	11.4 ± 1.0	12.0 ± 0.6[a]	14.0 ± 2.7	12.7 ± 1.5
osmolality (mOsm/l)	2210 ± 110	1727 ± 53[a]	1530 ± 120[a]	1340 ± 100[a,b]	1241 ± 71[a,b]

Results are presented as mean ± SEM

[a] significantly different from 4-month value, $p < 0.05$
[b] significantly different from 12-month value, $p < 0.05$
[c] significantly different from 24-month value, $p < 0.05$

them with invasion into brain tissue and the other animal displayed a small granular cell tumour, leukaemia and liver abnormalities. These three animals were excluded from the calculations in the pharmacokinetic/pharmacodynamic evaluations on the basis of these findings.

2. Pharmacokinetic/pharmacodynamic evaluation

2a. In vivo experiment

In figure 1 the blood concentration and anticonvulsant effect vs. time profiles after administration of oxazepam as an intravenous bolus dose of 12 mg in a typical 4-month-old male BN/BiRij rat are shown.

The mean oxazepam blood concentration vs. time profiles of the five age

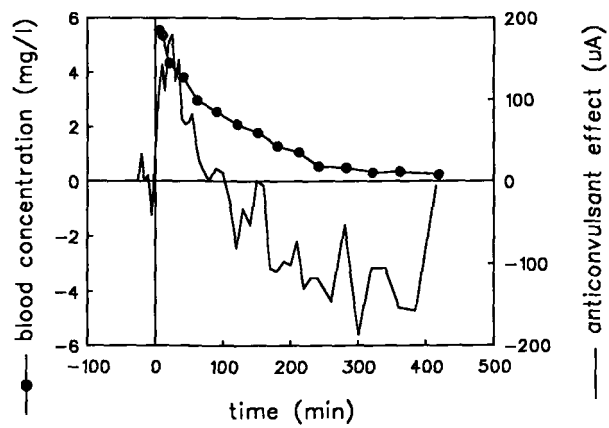


Figure 1. Blood concentration vs. time and anticonvulsant effect vs. time profiles after administration of oxazepam as an intravenous bolus dose of 12 mg/kg, in a typical 4-month-old male BN/BiRij rat. Anticonvulsant effect intensities (µA) were expressed as the elevation in seizure threshold over baseline level. At t = 0 min oxazepam was administered. The five effect values before t = 0 min represent the baseline determinations.

groups were identical (figure 2), which is reflected in comparable pharmacokinetic parameters in the age groups (table 2). Also the protein binding did not show age-related changes (table 2).

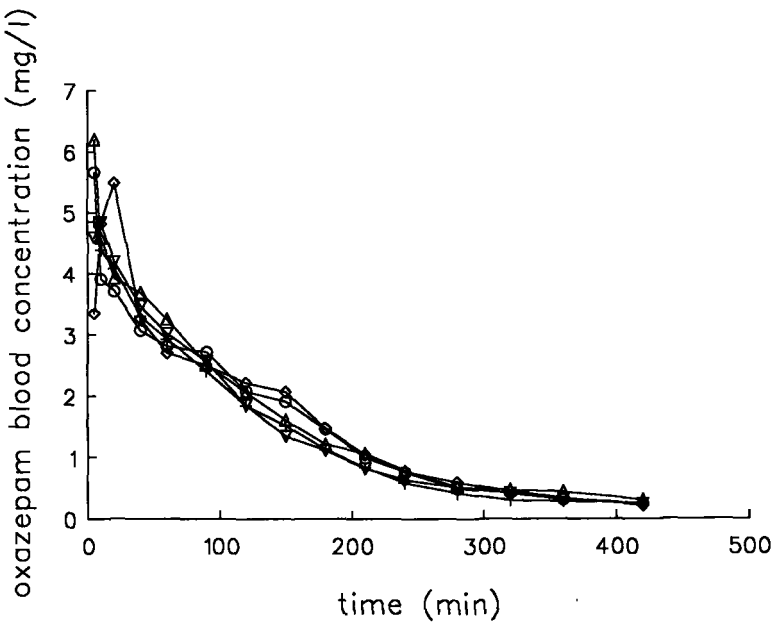


Figure 2. *Total blood concentration vs. time profiles after administration of* oxazepam as an intravenous bolus dose of 12 mg/kg, in male BN/BiRij rats of five different ages. The mean values of all animals in each age group are given (+ 4-month-old, △ 12-month-old, ○ 24-month-old, ◊ 31-month-old, ▽ 35-month-old).

Table 2. *The influence of ageing on the pharmacokinetic parameters of oxazepam in rats following an intravenous bolus dose of 12 mg/kg.*

age (month)	4	12	24	31	35
number of animals	8	8	7	6	7
total body clearance (ml/h.kg)	20.4 ± 0.9	15.2 ± 1.7	19.4 ± 1.1	18.7 ± 1.9	20.4 ± 2.5
volume of distribution (l/kg)	2.52 ± 0.19	2.40 ± 0.22	2.27 ± 0.20	2.42 ± 0.29	2.56 ± 0.15
elimination half-life (min)	95 ± 11	105 ± 6	81 ± 5	90 ± 7	92 ± 8
plasma protein binding (%)	88.4 ± 1.5	91.3 ± 1.2	93.6 ± 0.5	93.1 ± 1.0	92.4 ± 0.5

The results are presented as mean ± SEM

The anticonvulsant effect vs. time profile (figure 1) showed a biphasic effect, first an increase in seizure threshold, later followed by a transient drop below the baseline value. This drop below the baseline was not found in the 4-month-old male control animals, which only received the solvent.
Figure 3 shows the mean anticonvulsant effect vs. time profiles in the five age groups. The curves for the ages of 4, 12, 24 and 31 months are very similar: the shape is comparable, but the curves tend to move to higher anticonvulsant effect values with increasing age. The curve for the 35-month-old animals shows relatively high anticonvulsant effect values and a slower decline in anticonvulsant effect with time. Moreover, the effect does not drop below the baseline like in the other age groups.
The mean baseline seizure threshold value for all animals was 837 ± 40 μA (mean ± SEM; n=36). No systematic trend during ageing was found for the threshold values.
For all age groups the means of the highest anticonvulsant effect values in the individual animals were calculated (table 3). These values showed an age-related tendency to increase, with the value in the 35-month-old rats significantly higher than that in the 4-month-old animals.
In all individual animals the oxazepam blood concentration at which the anticonvulsant effect crossed the baseline value was determined graphically by eyeball fitting (table 3). In one animal in both the 4- and the 12-month-old group and three animals in both the 24- and the 31-month-old group, the effect did not drop below the baseline level but only returned to that value. In those animals the concentration at which the effect returned to the baseline was determined. No age-related change was found in the concentration at zero effect.

2b. In vitro benzodiazepine receptor binding characteristics
No age-related changes were found in the total number of specific binding sites and the apparent dissociation constant (table 4).

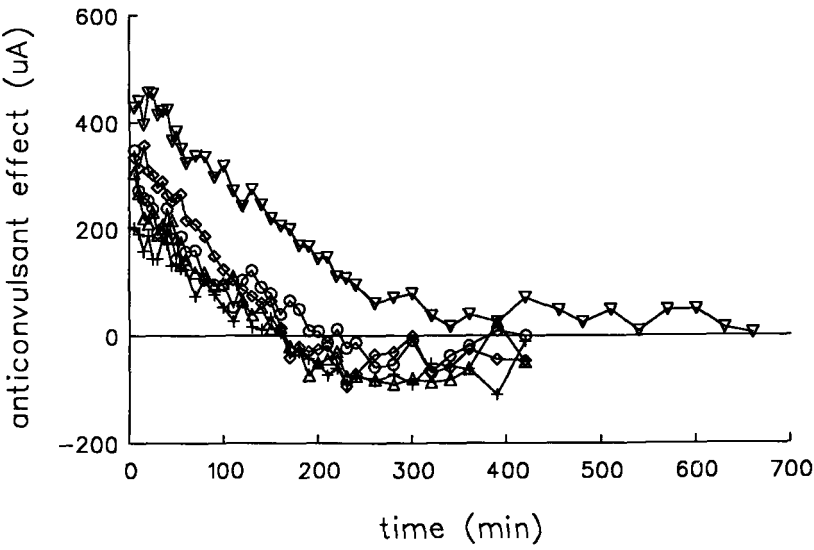


Figure 3. Anticonvulsant effect vs. time profiles after administration of oxazepam at an intravenous bolus dose of 12 mg/kg, in male BN/BiRij rats of five different ages. Anticonvulsant effect intensities (μA) were expressed as the elevation in seizure threshold over baseline level. The mean values of all animals in each age group are given (+ 4-month-old, Δ 12-month-old, o 24-month-old, ◊ 31-month-old, ▽ 35-month-old). The last eight values for the 35-month-old rats were measured in 5 out of the total of 7 animals.

Table 3. *The influence of ageing on the highest anticonvulsant effect values and the oxazepam blood concentrations at which the effect crossed the baseline value.*

age (month)	4	12	24	31	35
number of animals	8	8	7	6	7
highest anticonvulsant effect (μA)	262 ± 37	350 ± 37	365 ± 53	400 ± 15	549 ± 65[a]
concentration at baseline crossing (mg/l)	1.44 ± 0.23	1.52 ± 0.31	1.44 ± 0.30	1.39 ± 0.29	

The results are presented as mean ± SEM

[a] significantly different from 4-month value, $p < 0.05$

Table 4. The influence of ageing on the in vitro benzodiazepine receptor binding characteristics.

age (month)	4	12	24	31	35
number of animals	5	5	5	5	5
total number of specific binding sites (ng/mg)	0.55 ± 0.05	0.53 ± 0.22	0.54 ± 0.08	0.44 ± 0.05	0.52 ± 0.03
apparent dissociation constant (ng/ml)	4.0 ± 0.8	4.7 ± 0.9	3.3 ± 0.8	3.5 ± 0.8	5.8 ± 1.3

The results are presented as mean ± SEM

Discussion

In this investigation we determined simultaneously the time course of the plasma concentration and the anticonvulsant effect intensity of oxazepam in BN/BiRij rats of different age groups. The estimates of the pharmacokinetic parameters in young male BN/BiRij rats are comparable to those observed in female Wistar rats both with respect to the volume of distribution and clearance (Dingemanse et al., 1988, 1990). Interestingly, the pharmacokinetics of oxazepam were found not to change with increasing age, as is also the case in humans (Greenblatt et al., 1980; Murray et al., 1981; Ochs et al., 1981).

Literature data show that also for other benzodiazepines no age-related changes in volume of distribution and clearance were found in animal models of ageing, i.e. for clonazepam in mice, for desmethyldiazepam in rats and rabbits and for diazepam in rabbits (Tsang and Wilkinson, 1982; Barnhill et al., 1990). For diazepam, in rats, a small age-related increase in the volume of distribution was found with no change in clearance (Tsang and Wilkinson, 1982). In this respect it is interesting that for the metabolism of benzodiazepines both phase I and phase II reactions are important. The relative contribution of these two types of metabolic pathways to the overall metabolism is dependent on the chemical structure of the benzodiazepine. Phase I metabolism is known to decline during ageing for many drugs, whereas for phase II metabolism this only holds for a few drugs (Durnas et al., 1990). Since the metabolism of oxazepam in rats and humans is qualitatively different (in rats phase I metabolism is more important, in

humans phase II metabolism; Sisenwine et al., 1972; Greenblatt, 1981; Sisenwine and Tio, 1986) it is striking that the clearance of oxazepam does not change during ageing in both rats and humans.

Of considerable interest is the observation that at least in young rats, the time course of the pharmacological response shows a biphasic character: anticonvulsant effect followed by proconvulsant effect. This is in contrast to the situation in Wistar rats (Dingemanse et al., 1990). This profile (which appears not to be related to changes in the baseline seizure threshold) is highly suggestive for development of acute tolerance and/or withdrawal syndrome. This is also confirmed by the results of some pilot experiments, in which no proconvulsant effect was observed at low concentrations of oxazepam during slow intravenous infusion. Thus initially, high concentrations are required for the proconvulsant effect to occur (Stijnen et al., unpublished observations).

Lister and Nutt (1986) reported withdrawal phenomena in both mice and rats. These animals were sensitized to the proconvulsant action of a benzodiazepine receptor inverse agonist (FG 7142) following a single dose of lorazepam and were less sensitive to the effects of a second treatment with lorazepam (Lister and Nutt, 1986). Haefely (1986) mentioned tolerance for the anticonvulsant activity of benzodiazepines after subchronic treatment in rats.

In humans, the occurrence of acute tolerance has been reported for anti-anxiety effects and effects on psychomotor function of several benzodiazepines, including oxazepam (Bliding, 1974; Greenblatt et al., 1977; Macleod et al., 1977; Ellinwood et al., 1983, 1985, 1987). Withdrawal after cessation of benzodiazepine therapy is a well known clinical phenomenon (Greenblatt and Shader, 1978; Greenblatt et al., 1981; Ashton, 1984), also for oxazepam (Salzman et al., 1983). As a possible mechanism for the occurrence of withdrawal phenomena, Baldwin and File (1988) suggested increased production and release of an endogenous ligand for the benzodiazepine receptor, with inverse agonist properties. Novas et al. (1988) and Medina et al. (1989) actually reported an endogenous ligand with proconvulsant effects, being n-butyl-ß-carboline-3-carboxylate.

The proconvulsant component in the effect vs. time profile was found in our study in the ages of 4 to 31 months, but did not appear in the 35-month-old animals. Moreover, the anticonvulsant effect in the 35-month-old animals showed a slower decrease over time compared to the other ages. These two phenomena suggest disappearance of the tolerance and withdrawal phenomena in the old rats. In elderly humans, however, acute tolerance to the sedative effects of oxazepam (Dreyfuss et al., 1986) and to the effects of diazepam on neuromotor and cognitive functions (Nikaido et al., 1987) were reported. For benzodiazepines no data are available on tolerance to the anticonvulsant effect in elderly subjects. For a barbiturate (phenobarbital), however, in mice tolerance to the anticonvulsant effect of phenobarbital (after chronic dosing) was found in both young and old animals (Kitani et al., 1986). Concerning withdrawal phenomena after cessation of benzodiazepine therapy in the elderly, data from a spontaneous reporting system maintained by the Food and Drug Administration in the USA showed a much lower frequency of withdrawal in the older age group (Tanner et al., 1989), which agrees with our data in rats.

In the 35-month-old animals higher anticonvulsant effect values were measured relative to the younger animals. The underlying cause for these findings can be a change in pharmacokinetics (giving rise to higher concentrations at the site of action compared to younger animals), a decrease in protein binding or an increase in brain sensitivity.
Because both the oxazepam blood concentration vs. time profiles (figure 2) and the protein binding (table 2) did not change during ageing, it is not likely that the concentration of the drug at the site of action showed age-related changes. The age-related increase in the oxazepam concentration ratio brain/plasma in mice, as reported by Kitani et al. (1989), is probably due to higher aspecific binding to brain tissue, which does not influence the free concentration at the site of action.
An increased sensitivity during ageing is another likely explanation for the higher anticonvulsant effect values in the 35-month-old animals obtained at concentrations comparable to those in the younger animals. This is in agreement with the age-related increased sensitivity in mice for the anticonvulsant effect of several drugs (Kitani et al., 1984, 1985, 1987, 1988), including oxazepam (Kitani et al., 1989).
The higher brain sensitivity we found can be caused by changes in drug-

receptor interaction, post-receptor events or changes in homeostatic mechanisms (Danhof, 1989). In the in vitro receptor binding characteristics we did not observe age-related changes. Literature data on post-receptor events (GABA receptor binding and chloride channel functioning) do not show consistent evidence for age-related changes (De Blasi et al., 1982; Concas et al., 1988; Ito et al., 1988; Barnhill et al., 1990). A change in homeostatic mechanisms may be a reasonable cause for the higher brain sensitivity, because also the disappearance of the biphasic effect in the 35-month-old animals suggests a change in homeostatic mechanisms. Possibly the disappearance of the tolerance and withdrawal phenomena is the cause for the increased sensitivity. Moreover, it is generally accepted that the maintenance of homeostasis is impaired in the elderly (Shock, 1961, 1983; Bender, 1969; Crooks, 1983).

Summarizing, the biphasic pharmacological effect vs. time profiles (anticonvulsant effect followed by 'proconvulsant' effect) observed in the 4- to 31-month-old BN/BiRij rats after administration of an intravenous oxazepam bolus dose of 12 mg/kg are suggestive for acute tolerance development and withdrawal. The disappearance of this biphasic profile in the 35-month-old animals and the increase in brain sensitivity are compatible with a decreased efficiency of homeostatic control mechanisms in the elderly.

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References

Ashton, H.: Benzodiazepine withdrawal: an unfinished story. Br. Med. J. 288: 1135-1140, 1984.

Baldwin, H.A. and File, S.: Reversal of increased anxiety during benzodiazepine withdrawal: evidence for an anxiogenic endogenous ligand for the benzodiazepine receptor. Brain Res. Bull. 20: 603-606, 1988.

Barnhill, J.G., Greenblatt, D.J., Miller, L.G., Gaver, A., Hartmatz, J.S. and Shader, R.I.: Kinetic and dynamic components of increased benzodiazepine sensitivity in aging animals. J. Pharmacol.

Exp. Ther. 253: 1153-1161, 1990.

Bender, A.D.: Geriatric pharmacology. Age and its influence on drug action in adults. Drug Inf. Bull. 3: 153-158, 1969.

Bliding, A.: Effects of different rates of absorption of two benzodiazepines on subjective and objective parameters. Eur. J. Clin. Pharmacol. 7: 201-211, 1974.

Bowling, A.C. and De Lorenzo, R.J.: Micromolar affinity benzodiazepine receptors: identification and characterization in central nervous system. Science 216: 1247-1250, 1982.

Castleden, C.M., George, C.F., Marcer, D. and Hallett, C.: Increased sensitivity to nitrazepam in old age. Br. Med. J. 1, 10-12, 1977.

Concas, A., Pepitoni, S.J., Atsoggiu, T., Toffano, G. and Biggio, G.: Aging reduces the GABA dependent ^{36}Cl-flux in rat brain membrane vesicles. Life Sci. 43: 1761-1771, 1988.

Cook, P.J.: Benzodiazepine hypnotics in the elderly. Acta psychiatr. scand. 74: 149-158, 1986.

Crooks, J.: Aging and drug disposition - pharmacodynamics. J. Chron. Dis. 36: 85-90, 1983.

Danhof, M.: Does variability in drug disposition explain (all) variability in drug effects? in: Topics in Pharmaceutical Sciences 1989, D.D. Breimer, K.K. Midha and D.J.A. Crommelin, eds., Amsterdam Medical Press, Noordwijk, The Netherlands, 1989, pp 573-586.

De Blasi, A., Cotecchia, S. and Mennini, T.: Selective changes of receptor binding in brain regions of aged rats. Life Sci. 31: 335-340, 1982.

Dingemanse, J., Sollie, F.A.E., Breimer, D.D. and Danhof, M.: Pharmacokinetic modelling of the anticonvulsant response of oxazepam in rats using the pentylenetetrazol threshold concentration as pharmacodynamic measure. J. Pharmacokin. Biopharm. 16: 203-208, 1988.

Dingemanse, J., Voskuyl, R.A., Langemeijer, M.W.E., Postel-Westra, I., Breimer, D.D., Meinardi, H. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the anticonvulsant effect of oxazepam in individual rats. Br. J. Pharmacol. 99: 53-58, 1990.

Dreyfuss, D., Shader, R.I., Harmatz, J.S. and Greenblatt, D.J.: Kinetics and dynamics of single doses of oxazepam in the elderly: implications of absorption rate. J. Clin. Psychiatry 47: 511-514, 1986.

Durnas, C., Loi, C.M. and Cusack, B.J.: Hepatic drug metabolism and aging. Clin. Pharmacokin. 19: 359-389, 1990.

Ellinwood, E.H., Linnoila, M., Easler, M.E. and Molter, D.W.: Profile of acute tolerance to three sedative anxiolytics. Psychopharmacol. 79: 137-141, 1983.

Ellinwood, E.H., Heatherly, D.G., Nikaido, A.M., Bjornsson, T.D. and Kilts, C.: Comparative pharmacokinetics and pharmacodynamics of lorazepam, alprazolam and diazepam. Psychopharmacol. 86: 392-399, 1985.

Ellinwood, E.H., Nikaido, A.M., Heatherly, D.G. and Bjornsson, T.D.: Benzodiazepine pharmacodynamics: evidence for biophase rate limiting mechanisms. Psychopharmacol. 91: 168-174, 1987.

Greenblatt, D.J., Allen, M.D. and Shader, R.I.: Toxicity of high-dose flurazepam in the elderly. Clin. Pharmacol. Ther. 21: 355-361, 1977.

Greenblatt, D.J. and Shader, R.I.: Dependence, tolerance, and addiction to benzodiazepines: clinical and pharmacokinetic considerations. Drug Metab. Rev. 8: 13-28, 1978.

Greenblatt, D.J., Divoll, M., Harmatz, J.S. and Shader, R.I.: Oxazepam kinetics: effects of age and sex. J. Pharmacol. Exp. Ther. 215: 86-91, 1980.

Greenblatt, D.J.: Clinical pharmacokinetics of oxazepam and lorazepam. Clin. Pharmacokin. 6: 89-105, 1981.

Greenblatt, D.J., Divoll, M., Harmatz, J.S., MacLaughlin, D.S. and Shader, R.I.: Kinetics and clinical effects of flurazepam in young and elderly noninsomniacs. Clin. Pharmacol. Ther. 30: 475-486, 1981.

Haefely, W.: Biological basis of drug-induced tolerance, rebound, and dependence. Contribution of recent research on benzodiazepines. Pharmacopsychiat. 19: 353-361, 1986.

Hauser, W.A. and Kurland, L.T.: The epidemiology of epilepsy in Rochester, Minnesota, 1935-1967. Epilepia 16: 1-66, 1975.

Hollander-Jansen, M., Dingemanse, J., Langemeijer, M.W.E. and Danhof, M.: Relationship between receptor occupancy at 37°C and the anticonvulsant effect of flunitrazepam in rats. Pharm. Res. 6: 585-591, 1989.

Ito, Y., Ho, I.K. and Hoskins, B.: Cerebellar GABA and benzodiazepine receptor characteristics in young and aged mice. Brain Res. Bull. 21: 251-255, 1988.

Kanto, J, Kangas, L., Aaltonen, L. and Hilke, H.: Effect of age on the pharmacokinetics and sedative effect of flunitrazepam. Int. J. Clin. Pharmacol. Ther. Toxicol. 19: 400-404, 1981.

Kitani, K., Masuda, Y., Sato, Y., Kanai, S., Ohta, M. and Nokubo, M.: Increased anticonvulsant effect of phenytoin in aging BDF1 mice. J. Pharmacol. Exp. Ther. 229: 231-236, 1984.

Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: Increased anticonvulsant effect of phenobarbital with age in mice - a possible pharmacological index for brain aging. Life Sciences 37: 1451-1460, 1985.

Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: The effect of age on the adaptation of the brain to the anticonvulsant effect of phenobarbital in mice. Life Sciences 39: 483-491, 1986.

Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: Increased anticonvulsant effect of AD-810 (zonisamide) in aging BDF1 mice. Life Sciences 41: 1339-1344, 1987.

Kitani, K., Sato, Y., Kanai, S., Ohta, M., Nokubo, M. and Masuda, Y.: The neurotoxicity of phenobarbital and its effect in preventing pentylenetetrazole-induced maximal seizure in aging mice. Arch. Geront. Geriatr. 7: 261-271, 1988.

Kitani, K., Klotz, U., Kanai, S., Sato, Y., Ohta, M. and Nokubo, M.: Age-related differences in the coordination disturbance and anticonvulsant effect of oxazepam in mice. Arch. Geront. Geriatr. 9: 31-43, 1989.

Lister, R.G. and Nutt, D.J.: Mice and rats are sensitized to the proconvulsant action of a benzodiazepine-receptor inverse agonist (FG 7142) following a single dose of lorazepam. Brain Res. 379: 364-366, 1986.

Macleod, S.M., Giles, H.G., Patzalek, J.J. and Sellers, E.M.: Diazepam actions and plasma concentrations following ethanol ingestion. Eur. J. clin. Pharmacol. 11: 345-349, 1977.

Medina, J.H., Levi de Stein, M. and De Robertis, E.: n-[^{3}H]butyl-ß-carboline-3-carboxylate, a putative endogenous ligand, binds preferentially to subtype 1 of central benzodiazepine receptors. J. Neurochem. 52: 665-670, 1989.

Murray, T.G., Chiang, S.T., Koepke, H.H. and Walker, B.R.: Renal disease, age, and oxazepam kinetics. Clin. Pharmacol. Ther. 30: 805-809, 1981.

Nikaido, A.M., Ellinwood, E.H., Heatherly, D.G. and Dubow, D.: Differential CNS effects of diazepam in elderly adults. Pharmacol. Biochem. Behav. 27: 273-281, 1987.

Novas, M.L., Wolfman, C., Medina, J.H. and De Robertis, E.: Proconvulsant and 'anxiogenic' effects of n-butyl ß carboline-3-carboxylate, an endogenous benzodiazepine binding inhibitor from brain. Pharmacol. Biochem. Behav. 30: 331-336, 1988.

Ochs, H.R., Greenblatt, D.J. and Otten, H.: Disposition of oxazepam in relation to age, sex and cigarette smoking. Klin. Wochenschr. 59: 899-903, 1981.

Reidenberg, M.M., Levy, M., Warner, H., Couthino, C.B., Schwartz, M.A., Yu, G. and Cheripko, J.: Relationship between diazepam dose, plasma level, age, and central nervous system depression. Clin. Pharmacol. Ther. 23: 371-374, 1978.

Salzman, C., Shader, R.I., Greenblatt, D.J. and Harmatz, J.S.: Long v short half-life benzodiazepines in the elderly. Arch. Gen. Psychiatry 40: 293-297, 1983.

Shock, N.W.: Physiological aspects of aging in man. Ann. Rev. Physiol. 23: 97-122, 1961.

Shock, N.W.: Aging of physiological systems. J. Chron. Dis. 36: 137-142, 1983.

Sisenwine, S.F., Tio, C.O., Shrader, S.R. and Ruelis, H.W.: The biotransformation of oxazepam in man, miniature swine and rat. Arzneim. Forsch. 22: 682-687, 1972.

Sisenwine, S.F., and Tio, C.O.: The metabolic disposition of oxazepam in rats. Drug Metab. Disp. 14: 41-45, 1986.

Stijnen, A.M., Velders-van der Voort, S.H., Zurcher, C., Danhof, M. and Van Bezooijen, C.F.A.: The influence of concurrent disease on age-related changes in the pharmacokinetic-pharmacodynamic relationship of heptabarbital in rats. Submitted for publication.

Swift, C.G., Haythorne, J.M., Clarke, P. and Stevenson, I.H.: The effect of ageing on measured responses to single doses of oral temazepam. Br. J. clin. Pharmacol. 11, 413P-414P, 1981.

Swift, C.G., Ewen, J.M., Clarke, P. and Stevenson, I.H.: Responsiveness to oral diazepam in the elderly: relationship to total and free plasma concentrations. Br. J. clin. Pharmacol. 20, 111-118, 1985.

Swift, C.G., Swift, M.R., Ankier, S.I., Pidgen, A. and Robinson, J.: Single dose pharmacokinetics and pharmacodynamics of oral loprazolam in the elderly. Br. J. clin. Pharmacol. 20, 119-128, 1985a.

Tanner, L.A., Baum, C., Prela, C.-M. and Kennedy, D.L.: Spontaneous adverse reaction reporting in the elderly for 1986. J. Geriatric Drug Ther. 3, 31-54, 1989.

Troupin, A.S. and Johannessen, S.I.: Epilepsy in the elderly. A pharmacologic perspective. In: Epilepsy: current approaches to diagnosis and treatment. D.B. Smith, ed., Raven Press, Ltd., New York, 1990, pp 141-153.

Tsang, C.-F.C. and Wilkinson, G.R.: Diazepam disposition in mature and aged rabbits and rats. Drug Metab. Disp. 10: 413-416, 1982.

Voskuyl, R.A., Dingemanse, J. and Danhof, M.: Determination of the threshold for convulsions by direct cortical stimulation. Epilepsy Res. 3: 120-129, 1989.

Chapter 10

THE PHARMACODYNAMICS OF SODIUM VALPROATE IN AGEING BN/BIRIJ RATS

A.M. Stijnen[1,2], S. Hovinga[1,*], M.W.E. Langemeijer[1], A. Hoogerkamp[1], C.F.A. van Bezooijen[2] and M. Danhof[1]

[*]Deceased January 30, 1991

[1]Center for Bio-Pharmaceutical Sciences, Division of Pharmacology, University of Leiden, The Netherlands
[2]TNO Institute of Ageing and Vascular Research, Leiden, The Netherlands

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Summary

The aim of the present investigations was to study the influence of increasing age on the pharmacodynamics of sodium valproate in BN/BiRij rats, applying a threshold for electrically-induced localized seizure activity as a measure of the anticonvulsant effect. Seven groups of healthy male BN/BiRij rats were used, aged 3, 6, 12, 19, 25, 31 and 37 months. Plasma concentration vs. anticonvulsant effect relationships were determined during a continuous intravenous infusion of sodium valproate at a rate of 5.5 mg/min.kg. The infusion was terminated, when the anticonvulsant effect intensity had reached the maximum technical attainable level or, when this did not take place within three hours after the start of the infusion, at a total infusion time of three hours. The individual valproate plasma concentration vs. anticonvulsant effect profiles were interpolated using a least squares spline. The sodium valproate plasma concentrations needed to achieve an anticonvulsant effect intensity of 200, 400, 600, 800 and 1000 μA were calculated. A parallel shift in the

concentration vs. anticonvulsant effect relationships towards lower concentrations with increasing age was observed. This finding suggests increased sensitivity to the anticonvulsant effect of sodium valproate with increasing age in BN/BiRij rats.

Introduction

Sodium valproate is a broad spectrum antiepileptic drug, which is used in patients of all age groups from the very young to the very old (Perucca et al., 1984). Interestingly, clinical evidence indicates that, in contrast with many other drugs, the incidence of adverse drug reactions is lower in the elderly compared to the very young (Lancet editorial, 1980; Zafrani and Berthelot, 1982; Williams et al., 1984). A clear explanation for this observation is not available yet. A number of studies has focused on the age-related changes in pharmacokinetics of sodium valproate and thereby some changes have been detected (Bryson et al., 1983; Perucca et al., 1984; Bauer et al., 1985; Hall et al., 1985; Yu et al., 1985). At the level of pharmacodynamics, however, very little is known about age-related changes. Several studies have shown that the pharmacological effects of valproate are mediated, at least in part, via GABAergic inhibition (Van der Laan, 1979). In a study on the effect of sodium valproate in the GABA mediated effects on the hypothalamus-pituitary system, a decreased responsiveness was observed with increasing age (Monteleone et al., 1987). Thus apparently, increasing age is associated with a decreased sensitivity at the level of pharmacodynamics to at least some effects of sodium valproate. An interesting question is now, whether in the elderly there is also a decreased sensitivity to the anticonvulsant effect of sodium valproate. Recently, experimental techniques have been developed for pharmacodynamic investigations with anticonvulsants in small laboratory animals (Voskuyl et al., 1989; Dingemanse et al., 1990). In principle, these techniques allow the determination of concentration vs. anticonvulsant effect relationships in individual rats. This approach has also been applied successfully to the anticonvulsant effect of valproate (Hoogerkamp et al., in preparation).

The aim of the present study was therefore to investigate the concentration vs. anticonvulsant effect relationship of sodium valproate in ageing BN/BiRij rats (using seven different ages ranging from 3 to 37 months), applying the newly developed technique.

Materials and methods

Animals

Seven groups of male BN/BiRij rats (TNO Institute for Ageing and Vascular Research, Leiden, The Netherlands) of different ages (3, 6, 12, 19, 25, 31 and 37 months) were used for the main experiment. The 10, 50 and 90 % survival ages of the male BN/BiRij rats are 38.1, 31.7 and 22.8 months, respectively. During the period in which the experiments were performed, the rats were kept solitary in Makrolon cages and in a normal 12-hr light-dark cycle (light between 7.00 A.M. and 7.00 P.M.). The temperature was maintained at 22-23 °C. They were allowed free access to water (acidified, pH 3-4) and food (Standard diet for Rat, Mouse and Hamster, AM 1410, Hope Farms, Woerden, The Netherlands).

Chemicals

Sodium valproate was kindly donated by Labaz-Sanofit (Maassluis, the Netherlands).

Animal experiments

1. Clinical biochemical/pathological evaluation

In order to be able to determine the health status of the animals, several clinical biochemical parameters were measured. Blood concentrations of urea nitrogen, aspartate aminotransferase, alanine aminotransferase, glucose, total plasma protein and albumin concentration, 24 hour urine production, urine osmolality, and creatinine clearance were determined as described before (Stijnen et al., submitted; chapter 6).

After the pharmacokinetic/pharmacodynamic evaluation, the animals were sacrificed, the liver was weighed and the heart, the lungs, the kidneys, the liver, the brain and macroscopically visible abnormalities were evaluated by a pathologist, who was unaware of the outcome of the pharmacokinetic/pharmacodynamic evaluation.

2. Pharmacokinetic/pharmacodynamic evaluation

The threshold to induce localized seizure activity was used as a measure of the anticonvulsant effect of sodium valproate. This threshold was determined by measuring the convulsant stimulus corresponding to the (visually determined) point at which clonic forelimb activity started. As convulsant stimulus, a bipolar pulse train (duration of one pulse 2 ms, frequency 50 pulses per s) of linearly increasing amplitude (0-2400 μA in 20 s) was used, applied to two electrodes in the skull over the motor area of the frontoparietal cortex. These electrodes were implanted three weeks before the experiment. Since the threshold of the anticonvulsant stimulus, which would be measured repeatedly in the experiment, decreases in the initial test sessions, this threshold was determined twice daily during the two weeks before the actual experiment in order to achieve a stable baseline (Voskuyl et al., 1989).

One day before the experiment, a cannula was implanted in the right jugular vein under light halothane anaesthesia. Just before the administration of sodium valproate the threshold to induce localized seizure activity was determined five times; the mean threshold value was used as the baseline level. Anticonvulsant effect intensities after administration of sodium valproate were expressed as the elevation in threshold over this baseline level.

Sodium valproate was administered as a continuous intravenous infusion at a rate of 5.5 mg/min.kg. The seizure threshold was determined every 3 minutes and 14 blood samples of 100 μl were taken from an incision in the tail during the total three hours of the infusion. If the threshold reached the maximum level, attainable by the apparatus used, before these three hours of infusion, the infusion was stopped at that moment. The cerebrospinal fluid, the brain and the blood were collected at the end of the infusion, in order to determine the distribution of sodium valproate in the different age groups.

Drug analysis

Sodium valproate concentrations in plasma, plasma ultrafiltrate, cerebrospinal fluid and brain tissue were determined with a gas chromatographic (GC) method after an extraction procedure. To 50 μl plasma or plasma ultrafiltrate or 2.5 to 55 μl cerebrospinal fluid, 100 μl 1N HCl was added and 0.08 μl cyclohexane carboxylic acid as internal standard. The mixture was extracted for 20 seconds with 1 ml freshly distilled petroleum ether. Of the petroleum

ether layer 1 µl was injected into the GC. To determine the sodium valproate concentration in brain, one hemisphere was weighed and homogenized in distilled water (total volume of hemisphere and water 3 ml). To 0.5 ml of the homogenate, 0.5 ml 6N HCl was added and the mixture was extracted for 20 seconds with 3 ml of petroleum ether, containing 0.77 µl cyclohexane carboxylic acid. 0.2 ml of the organic layer was diluted with 3 ml petroleum ether and 1 µl of this solution was injected into the GC.
A Hewlett Packard Model 5710A gas chromatograph, equipped with a flame ionization detector and a split inlet injection system. A fused silica capillary column (10 m x 0.53 mm ID) with a cross-linked FFAP stationary phase was used. Temperatures and chromatographic conditions were as follows: injection port, 250 °C; oven, 200 °C; detector, 140 °C; helium as carrier at 20 ml/min through the column and 30 ml/min through the detector; hydrogen, 30 ml/min; air, 270 ml/min. Retention times were 1.2 and 2.3 minutes for valproate and cyclohexane carboxylic acid, respectively. Chromatograms were recorded on a Hewlett Packard 3390A reporting integrator.
Calibration curves were linear with $r > 0.996$. Detection limit was about 1 mg/l, coefficients of variation less than 5% for plasma, plasma ultrafiltrate and cerebrospinal fluid and less than 7% for brain tissue (n=5).
Protein binding of sodium valproate was determined by means of ultrafiltration using the Amicon MPS-1 system (Grace B.V., Rotterdam, The Netherlands).

Data analysis

1. Pharmacodynamics
The plasma concentration vs. anticonvulsant effect curves were constructed by combining the concentration vs. time and anticonvulsant effect vs. time profiles, obtained during continuous infusion of sodium valproate. To determine the concentrations at the time points were no blood sample was taken, linear interpolation of the concentration vs. time profiles was applied.
Because we only wanted to study the pharmacodynamics in a situation of equilibrium between plasma and the site of action, a separate experiment was performed, in which the distribution of sodium valproate between plasma and cerebrospinal fluid was determined also during infusion of sodium valproate at a rate of 5.5 mg/kg.min, in order to have an indication about the distribution between plasma and the site of action. Since the equilibrium between plasma and cerebrospinal fluid was reached after 30 minutes of infusion, only the

data gathered after 30 minutes were used.
The plasma concentration vs. anticonvulsant effect curves were interpolated using a least squares spline with a variable number of knots (Veng-Pederson et al., in press). The sodium valproate concentrations needed to achieve an anticonvulsant effect of 200, 400, 600, 800 and 1000 μA were determined.

2. Statistics
Differences between age groups in clinical biochemical indices and in sodium valproate concentrations needed to achieve an anticonvulsant effect of 200, 400, 600, 800 and 1000 μA were statistically evaluated by one-way analysis of variance followed by the Student's t-test with Bonferroni correction. Bartlett's test was used to assess homogeneity of variances. In case of nonhomogeneity of variances the Welch test and the multiple Welch test were used. P-values lower than 0.05 were judged to be significant.

Results

1. Clinical biochemical/pathological evaluation
The results of the clinical biochemical indices are shown in table 1. The blood alanine aminotransferase was fluctuating during ageing with a decrease in the 19-, 25- and 37-month-old rats; the same holds for the blood urea nitrogen with a decrease in the 19-, 25- and 31-month-old rats. The creatinine clearance appeared to be decreased in the 25-month-old rats, the urine production in the 19-month-old ones and the osmolality in the 31- and 37-month-old rats.
The pathological evaluation was performed in an attempt to differentiate between changes due to physiological ageing and changes as result of concomitant pathology. One 25-month-old animal showed moderate to severe renal lesions (tubular atrophy, cast formation, moderate diffuse glomerulopathy) and was excluded from the evaluations on the basis of these findings.

2. Pharmacokinetic/pharmacodynamic evaluation
The baseline seizure threshold values (table 2) appeared to be independent of age.
In figure 1 the plasma concentration vs. anticonvulsant effect profile in a typical 3-month-old animal is shown. The sodium valproate plasma

Table 1. Effect of age on selected clinical biochemical indices.

age (month)	3	6	12	19	25	31	37
number of animals	9	10	10	10	9	8	4
body weight (g)	200 ± 9	309 ± 7[a]	362 ± 9[a,b]	416 ± 17[a,b]	439 ± 13[a,b,c]	380 ± 10[a,b,e]	368 ± 18[a]
liver weight (g)	7.1 ± 0.4	7.6 ± 0.1	8.7 ± 0.3	9.4 ± 0.4 (n=9)	10.3 ± 0.7	10.7 ± 1.1	9.5 ± 0.7
liver wt/ body wt (%)	3.62 ± 0.24	2.47 ± 0.04	2.41 ± 0.08	2.30 ± 0.07 (n=9)	2.37 ± 0.37	2.78 ± 0.22	2.57 ± 0.12
blood aspartate aminotransferase (I.U./l)	94.2 ± 7.6	100.7 ± 7.5	97.1 ± 8.1	96 ± 12	101 ± 17	73.5 ± 4.1	77.8 ± 8.8
blood alanine aminotransferase (I.U./l)	46.2 ± 2.0	41.0 ± 1.8	42.3 ± 1.7	34.1 ± 2.1[a]	32.4 ± 2.2[a,c]	36.5 ± 2.2	32.3 ± 2.1[a]
plasma albumin (mg/ml)	35.2 ± 4.0	29.4 ± 1.6	29.9 ± 1.6	27.5 ± 1.3	27.2 ± 1.4	27.4 ± 1.8	28.4 ± 0.9
plasma total protein (mg/ml)	72.9 ± 1.3	83.4 ± 2.8	80.5 ± 0.6	83.3 ± 1.5	79.9 ± 1.3	80.2 ± 0.7	76.3 ± 1.9
blood glucose (mmol/l)	5.3 ± 0.3	5.4 ± 0.1	4.9 ± 0.2	5.2 ± 0.2	4.8 ± 0.2	5.4 ± 0.3	5.1 ± 0.3
blood urea nitrogen (mmol/l)	8.0 ± 0.4	9.3 ± 0.3	8.1 ± 0.3	7.1 ± 0.3[b]	6.7 ± 0.4[b]	7.3 ± 0.2[b]	7.5 ± 0.3
creatinine clearance (ml/h.kg)	213 ± 16	171 ± 14	168 ± 11	154 ± 10	131 ± 14[a]	180 ± 20	138 ± 13
urine production (ml/24 hrs)	10.5 ± 1.2	10.1 ± 1.1	11.5 ± 0.4	7.9 ± 0.8[c]	8.6 ± 1.1	13.9 ± 1.9	9.2 ± 2.1
osmolality (mOsm/l)	1690 ± 130	1940 ± 100	1541 ± 49	1810 ± 120	1700 ± 110	1340 ± 68[b]	1267 ± 41[b,c,d]

Results are presented as mean ± SEM

[a] significantly different from 3-month value, $p < 0.05$
[b] significantly different from 6-month value, $p < 0.05$
[c] significantly different from 12-month value, $p < 0.05$
[d] significantly different from 19-month value, $p < 0.05$
[e] significantly different from 25-month value, $p < 0.05$

concentrations needed to achieve an anticonvulsant effect intensity of 800 and 1000 μA showed a decrease of about 40% between the ages of 6 and 37 months (figure 2 and table 2). The concentrations needed to achieve an anticonvulsant effect of 200, 400 and 600 μA showed a tendency to decrease during ageing, which did not reach statistical significance. These results show that the concentration vs. anticonvulsant effect curve shows a parallel shift to lower concentrations during ageing.

The distribution of sodium valproate between plasma (free drug and protein

bound drug), cerebrospinal fluid and brain tissue at the end of the continuous infusion did not show differences between the age groups (table 3).

Table 2. *Effect of age on baseline seizure threshold values and pharmacodynamics of sodium valproate.*

age (month)	3	6	12	19	25	31	37
number of animals	9	10	10	10	8	8	4
baseline seizure threshold (μA)	723 ± 89	674 ± 36	687 ± 83	686 ± 42	643 ± 89	723 ± 40	740 ± 56
$conc._{200}$ (mg/l)[A]	592 ± 84	582 ± 42	511 ± 74	452 ± 52	459 ± 54	449 ± 43	353 ± 83
$conc._{400}$ *(mg/l)*	*862 ± 67*	*893 ± 58*	*802 ± 82*	*777 ± 50*	*713 ± 54*	*642 ± 40*	*505 ± 49*[b]
$conc._{600}$ (mg/l)	1048 ± 67	1073 ± 65	1000 ± 77 (n=9)	986 ± 51	901 ± 65	786 ± 40	664 ± 37
$conc._{800}$ (mg/l)	1163 ± 58	1204 ± 69	1153 ± 53 (*n*=9)	1118 ± 54	1036 ± 78	889 ± 35[a,b,c]	744 ± 47[a,b,c,d]
$conc._{1000}$ (mg/l)	1287 ± 47 (*n=7*)	1329 ± 79	1303 ± 47 (*n=8*)	1249 ± 62 (*n=9*)	1160 ± 90	996 ± 33[a,c]	811 ± 62[a,b,c,d]

Results are presented as mean ± SEM

[A] $conc._{200}$ = concentration needed to achieve an anticonvulsant effect consisting of an elevation in seizure threshold over baseline level of 200 μA

[a] significantly different from 3-month value, $p < 0.05$
[b] significantly different from 6-month value, $p < 0.05$
[c] significantly different from 12-month value, $p < 0.05$
[d] significantly different from 19-month value, $p < 0.05$

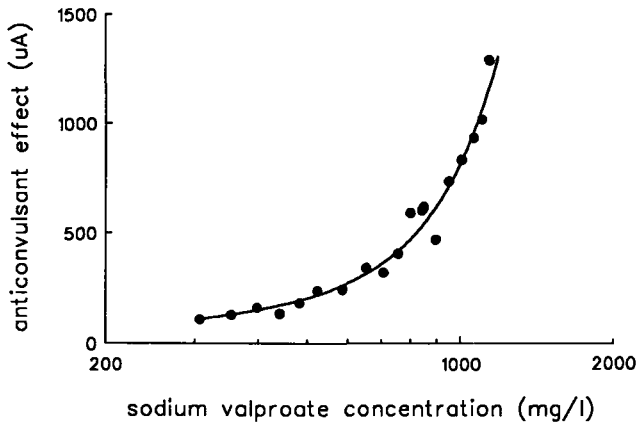

Figure 1. Anticonvulsant effect vs. plasma concentration profile measured during continuous sodium valproate intravenous infusion at a rate of 5.5 mg/min.kg in a typical 3-month-old animal. Anticonvulsant effect intensities (μA) were expressed as the elevation in seizure threshold over baseline level. The line through the data points represents the least squares spline used for interpolation.

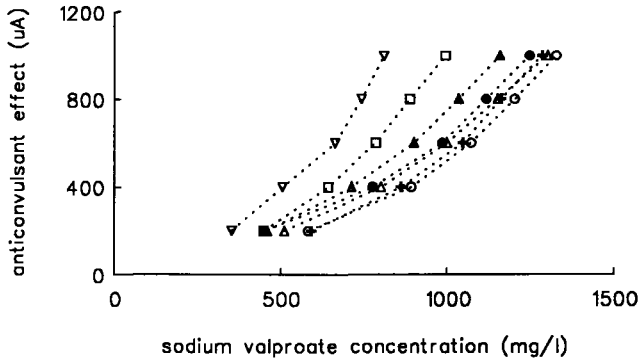

Figure 2. The 'threshold' plasma concentrations needed to achieve anticonvulsant effect intensities of 200, 400, 600, 800 and 1000 μA for the different age groups (+ 3-month-old, o 6-month-old, △ 12-month-old, ● 19-month-old, ▲ 25-month-old, □ 31-month-old, ▽ 37-month-old).

Discussion

Sodium valproate is widely used in the treatment of various forms of epilepsy. The mechanism of the anticonvulsant effect is not completely understood. There is evidence, however, that this is mediated by an interaction at the level of GABAergic neurotransmission. It has for example been demonstrated that sodium valproate is able to inhibit GABA degradation (Van der Laan, 1979) and to increase GABA release from nerve endings (Löscher and Siemes, 1984). Also there appears to be a direct interaction with the GABA-benzodiazepine receptor complex (Liljequist and Engel, 1984).
Recently it has been reported that there is an age-related decrease in the sensitivity of the hypothalamic-pituitary system to sodium valproate, resulting in a decreased pituitary hormone secretion (Monteleone et al., 1987). Because GABA plays a role in the regulation of pituitary hormone secretion (Racagni et al., 1982), it seems likely that the reduced hypothalamus-pituitary response with increasing age is the result of changes at the level of the GABAergic transmission (Monteleone et al., 1987).
The purpose of the present study was to determine, whether increasing age

Table 3. *Effect of age on relative distribution of sodium valproate.*

age (month)	3	6	12	19	25	31	37
number of animals	8	10	9	10	8	8	4
conc. csf/ conc. plasma free[A]	0.71 ± 0.02	0.73 ± 0.02	0.65 ± 0.02	0.77 ± 0.08	0.74 ± 0.01	0.75 ± 0.18	0.74 ± 0.08
conc. csf/ conc. plasma total	0.65 ± 0.02	0.67 ± 0.03 (n=9)	0.61 ± 0.02 (n=8)	0.66 ± 0.06 (n=9)	0.71 ± 0.02 (n=7)	0.65 ± 0.04 (n=6)	0.67 ± 0.07
conc. csf/ conc. brain	1.86 ± 0.06 (n=7)	2.08 ± 0.20 (n=9)	2.12 ± 0.08 (n=7)	1.88 ± 0.10 (n=9)	1.94 ± 0.09 (n=7)	1.95 ± 0.09 (n=6)	2.40 ± 0.16
plasma protein binding (%)	9.0 ± 1.9	10.7 ± 2.3	8.2 ± 2.3	13.9 ± 3.0	8.9 ± 2.6	9.4 ± 3.0	9.6 ± 0.9
conc. brain/ conc. plasma free	0.38 ± 0.01 (n=7)	0.38 ± 0.03	0.31 ± 0.01	0.40 ± 0.03	0.39 ± 0.02	0.39 ± 0.02	0.31 ± 0.01
conc. brain/ conc. plasma total	0.34 ± 0.01 (n=7)	0.36 ± 0.02	0.29 ± 0.01	0.34 ± 0.02	0.37 ± 0.02	0.34 ± 0.01	0.28 ± 0.01

Results are presented as mean ± SEM

[A] conc. = concentration; csf = cerebrospinal fluid

is also associated with a decrease in the sensitivity to the anticonvulsant effect of valproate. For that purpose, sodium valproate was given as a slow intravenous infusion and the intensity of the anticonvulsant effect was measured repeatedly on the basis of the direct cortical stimulation model (Voskuyl et al., 1989). Since also the plasma concentration of sodium valproate was measured at several time points, concentration vs. anticonvulsant effect profiles could be determined in individual animals. Between the ages of 6 and 37 months, a parallel shift in the concentration vs. anticonvulsant effect relationship towards lower concentrations was found. This finding suggests increased sensitivity to the anticonvulsant effect with increasing age.

To be able to conclude from the age-related parallel shift in the concentration vs. anticonvulsant effect relationships that the brain sensitivity increases during ageing, it has to be excluded that the pharmacokinetic factors protein binding, distribution of the drug between plasma and the site of action in the brain and active metabolites do not interfere. The protein binding appeared not to be changing during ageing (table 3). We only used the concentration and anticonvulsant effect data, gathered after 30 minutes of infusion, when an equilibrium was reached between cerebrospinal fluid and plasma, and thus probably also between plasma and at the site of action (Stijnen et al., unpublished observations). Therefore, and because the ratios between the concentrations in plasma, cerebrospinal fluid and brain tissue at the end of the infusion were not age-related (table 3), it is unlikely that the age-related parallel shift in the concentration vs. anticonvulsant effect relationship is due to a distribution phenomenon. Regarding the role of active metabolites, it is important that metabolism of sodium valproate is very complex and not completely elucidated (Chapman et al., 1982; Eadie, 1991; Li et al., 1991). Therefore, we determined the concentration vs. anticonvulsant relationships during intravenous infusion, in order to minimize metabolite concentrations. Because we did not measure metabolites in our experiment, we cannot exclude the possibility that age-related changes in metabolism contribute to the change in the plasma concentration vs. anticonvulsant effect relationship. However, sodium valproate itself is reported to be responsible for more than 90% of the activity, and in mice, the anticonvulsant potencies of the metabolites were reported to be lower than the mother compound (Chapman et al., 1982). If this also holds for the BN/BiRij rats, it would be unlikely that the age-related parallel shift of the concentration vs. anticonvulsant effect

relationships towards lower concentrations could be attributed to a change in metabolism, because for metabolites with a higher activity than the mother compound parallel shift towards lower concentration of the mother compound would be expected.

These considerations show that an increased brain sensitivity is the most likely cause for the parallel shift in the concentration vs. anticonvulsant effect relationship towards lower concentrations with increasing age.

The age-related increase in sensitivity to the anticonvulsant effect is in agreement with the age-related increase in sensitivity for several anticonvulsants in mice (Kitani et al., 1984, 1985, 1987, 1988, 1989). However, it is not in agreement with the decrease in hypothalamic-pituitary responsiveness to sodium valproate in humans (Monteleone et al., 1987) and the higher frequency of adverse effects in young patients compared to older ones (Lancet editorial, 1980; Zafrani and Berthelot, 1982; Williams et al., 1984). This suggests that the three effects (anticonvulsant, hypothalamic-pituitary and the adverse effects) are accomplished by different mechanisms. It is possible that the anticonvulsant effect of sodium valproate dose not only include GABAergic effects, but maybe also other mechanisms are involved.

Concluding it can be stated that age influences the pharmacodynamics of sodium valproate in male BN/BiRij rats, resulting in an increased, rather than a decreased, sensitivity to the anticonvulsant effect during ageing.

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References

Bauer, L.A., Davis, R., Wilensky, A., Raisys, V. and Levy, R.H.: Valproic acid clearance: unbound fraction and diurnal variation in young and elderly adults. Clin. Pharmacol. Ther. 37: 697-700, 1985.

Bryson, S.M., Verma, N., Scott, P.J.W. and Rubin, P.C.: Pharmacokinetics of valproic acid in young and elderly subjects. Br. J. clin. Pharmacol. 16: 104-105, 1983.

Chapman, A., Keane, P.E., Meldrum, B.S., Simiand, J. and Vernieres, J.C.: Mechanism of anticonvulsant action of valproate. Prog. Neurobiol. 19: 315-359, 1982.

Dingemanse, J., Voskuyl, R.A., Langemeijer, M.W.E., Postel-Westra, I., Breimer, D.D., Meinardi, H. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the anticonvulsant effect of oxazepam in individual rats. Br. J. Pharmacol. 99: 53-58, 1990.

Eadie, M.J.: Formation of active metabolites of anticonvulsant drugs. A review of their pharmacokinetic and therapeutic significance. Clin. Pharmacokin. 21: 27-41, 1991.

Editorial: Sodium valproate and the liver. Lancet 2: 1119-1120, 1980.

Hall, K., Otten, N., Johnston, B., Irvine-Meek, J., Leroux, M. and Seshia, S.: A multivariable analysis of factors governing the steady-state pharmacokinetics of valproic acid in 52 young epileptics. J. Clin. Pharmacol. 25: 261-268, 1985.

Kitani, K., Masuda, Y., Sato, Y., Kanai, S., Ohta, M. and Nokubo, M.: Increased anticonvulsant effect of phenytoin in aging BDF1 mice. J. Pharmacol. Exp. Ther. 229: 231-236, 1984.

Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: Increased anticonvulsant effect of phenobarbital with age in mice - a possible pharmacological index for brain aging. Life Sciences 37: 1451-1460, 1985.

Kitani, K., Sato, Y., Kanai, S., Nokubo, M., Ohta, M. and Masuda, Y.: Increased anticonvulsant effect of AD-810 (zonisamide) in aging BDF1 mice. Life Sciences 41: 1339-1344, 1987.

Kitani, K., Sato, Y., Kanai, S., Ohta, M., Nokubo, M. and Masuda, Y.: The neurotoxicity of phenobarbital and its effect in preventing pentylenetetrazol-induced maximal seizure in aging mice. Arch. Geront. Geriatr. 7: 261-271, 1988.

Kitani, K., Klotz, U., Kanai, S., Sato, Y., Ohta, M. and Nokubo, M.: Age-related differences in the coordination disturbance and anticonvulsant effect of oxazepam in mice. Arch. Geront. Geriatr. 9: 31-43, 1989.

Li, J., Norwood, D.L., Mao, L.-F. and Schulz, H.: Mitochondrial metabolism of valproic acid. Biochemistry 30: 388-394, 1991.

Liljequist, S. and Engel, J.A.: Reversal of the anti-conflict action of valproate by various GABA and benzodiazepine antagonists. Life Sci. 34: 2525-2533, 1984.

Löscher, W. and Siemes, H.: Valproic acid increases gamma-aminobutyric acid in CSF of epileptic children. Lancet II: 225, 1984.

Monteleone, P., Iovino, M., Orio, F. and Steardo, L.: Impaired growth hormone response to sodium valproate in normal aging. Psychopharmacol. 91: 10-13, 1987.

Perucca, E., Grimaldi, R., Gatti, G., Pirracchio, S., Crema, F. and Frigo, G.M.: Pharmacokinetics of valproic acid in the elderly. Br. J. clin. Pharmacol. 17: 665-669, 1984.

Racagni, G., Apud, J.A., Cocchi, D., Locatelli, V. and Müller, E.E.: GABAergic control of anterior pituitary hormone secretion. Life Sci. 31: 823-838, 1982.

Stijnen, A.M., Velders-van de Voort, S.H., Zurcher, C., Danhof, M. and Van Bezooijen, C.F.A.: The influence of concurrent disease on age-related changes in the pharmacokinetic-pharmacodynamic relationship of heptabarbital in rats. Submitted for publication.

Van der Laan, J.W., De Boer, T. and Bruinvels, J.: Di-n-propylacetate and GABA degradation. Preferential inhibition of succinic semialdehyde dehydrogenase and indirect inhibition of GABA-transaminase. J. Neurochem. 32: 1769-1780, 1979.

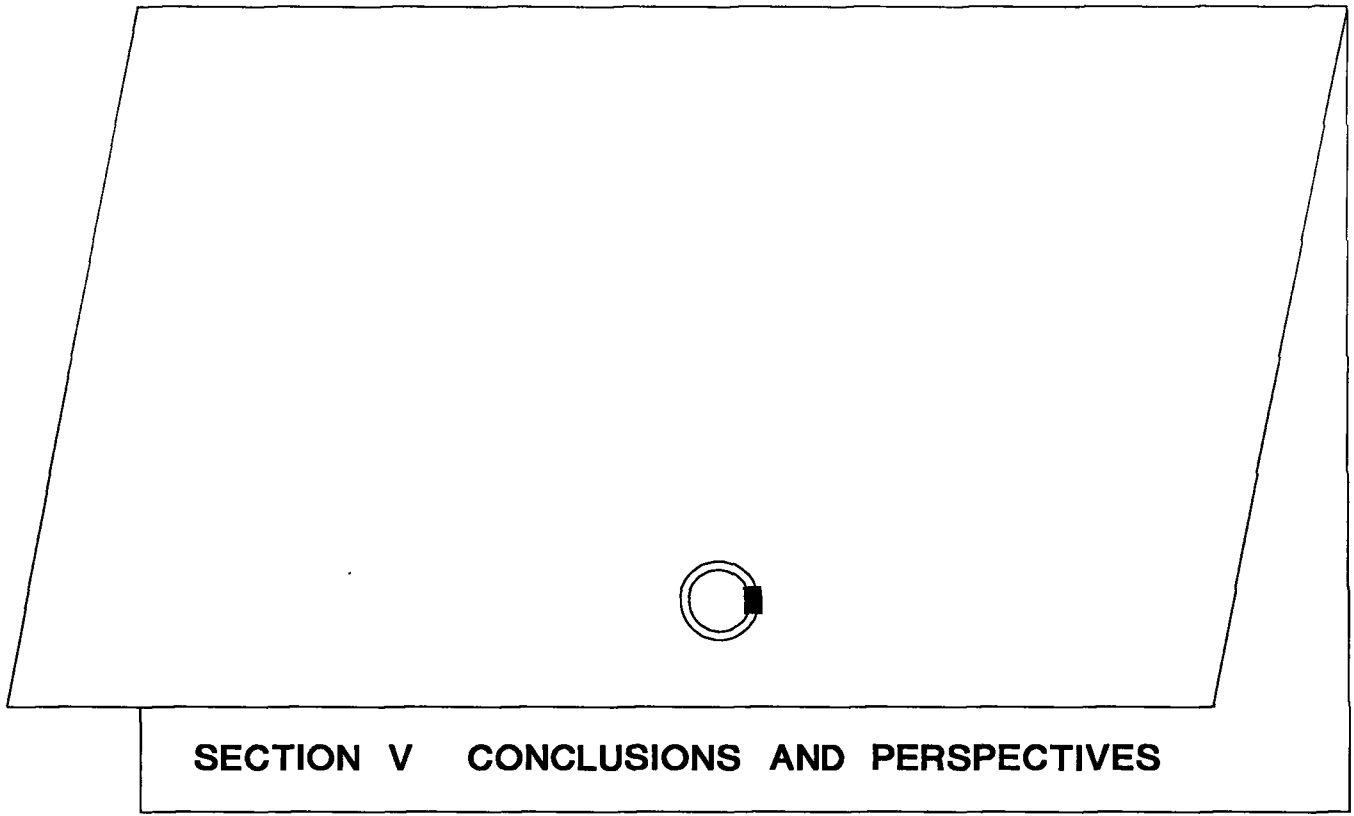
Veng-Pederson, P., Mandema, J.W. and Danhof, M.: Biophase equilibration times. J. Pharm. Sci., in press.

Voskuyl, R.A., Dingemanse, J. and Danhof, M.: Determination of the threshold for convulsions by direct cortical stimulation. Epilepsy Res. 3: 120-129, 1989.

Williams, C.A., Tiefenbach, S. and McReynolds, J.W.: Valproic acid-induced hyperammonemia in mentally retarded adults. Neurology 34: 550-553, 1984.

Yu, H.Y., Sugiyama, Y. and Hanano, M.: Changes in pharmacokinetics of valproic acid in guinea pigs from birth to maturity. Epilepsia 26: 243-251, 1985.

Zafrani, E.S. and Berthelot, P.: Sodium valproate in the induction of unusual hepatotoxicity. Hepatology 2: 648-649, 1982.



SECTION V CONCLUSIONS AND PERSPECTIVES

Chapter 11

THE INFLUENCE OF AGEING ON THE PHARMACODYNAMICS OF SEDATIVE AND ANTICONVULSANT DRUGS IN RATS

CONCLUSIONS AND PERSPECTIVES

The primary aim of the investigations described in this thesis was to study the influence of increasing age on the pharmacodynamics of drugs acting on the central nervous system. Thereby the contributions of concomitant pathology and of pharmacokinetic interfering factors were excluded. The rationale for these studies was the hypothesis, that age-related changes in pharmacodynamics may be important to explain, at least partially, the decreased dose requirement in the elderly that is observed for many drugs with a depressant effect on the central nervous system.

In order to be able to account rigorously for age-associated pathology and for pharmacokinetic factors, the experiments were performed in an animal model of ageing. Male BN/BiRij rats of four to seven different age groups, covering the age range between 3 and 37 months were used for this purpose. Three different effects on the central nervous system were investigated using model drugs: 1. the anaesthetic effect of two barbiturates, phenobarbital and heptabarbital, applying the technique of loss of righting reflex; 2. the EEG effect of heptabarbital and the benzodiazepine midazolam; 3. the anticonvulsant effect of the benzodiazepine oxazepam and of sodium valproate, using a direct cortical stimulation technique.

In the following, first several gerontological aspects of the studies described in this thesis will be discussed. Subsequently, pharmacological aspects will be discussed.

Gerontological aspects:

1. Influence of pathology

Ageing is associated with an increasing incidence of pathology (Burek, 1978; Brody and Schneider, 1986; Hollander et al., 1990). In order to be able to differentiate between the influence of ageing *per se* and the influence of age-related pathology on pharmacodynamics, all animals were subjected to an extensive pathological evaluation. This evaluation was performed by an independent pathologist on the basis of post-mortem tissue examination and additional clinical biochemical data. In chapter 6, it was shown that including and excluding diseased animals from the pharmacokinetic and pharmacodynamic investigations has major implications with regard to the conclusions.

Overviewing all investigations, described in this thesis, 8% of the animals (26 animals out of a total of 334 animals) had to be excluded from the evaluations because of pathological abnormalities. In table 1 the distribution of the diseased animals over the age groups is shown. In agreement with literature data (Burek, 1978; Hollander et al., 1990), we found an increase in the incidence of pathology with increasing age, with in particular the oldest animals being affected. The diseased animals displayed various kinds of pathology. The data give the impression, that the kind of pathology may sometimes be cohort-related. Hydronephrosis and pituitary tumours for example were found in a number of rats of only one cohort (the 31-month-old rats in chapter 6), whereas these abnormalities were not seen in any other cohort included in the investigations described in this thesis. All three diseased 35-month-old animals in the investigations in chapter 9 showed granular cell tumours, whereas in the other studies this abnormality was found

Table 1. *Number of diseased animals in the different age groups.*

age (month)	3-4	5-7	12-15	19	21-26	28-31	34-37	3-37
n (diseased)	1	0	3	0	4	9	9	26
n (total)	53	59	63	10	58	56	35	334
% diseased	2	0	5	0	7	16	26	8

to be extremely rare. Only three other animals from different studies were found to be affected. Liver abnormalities were frequently observed in the 36-month-old rats in chapter 8 and only in one other animal. These results show that it is important to include all relevant organs in the pathological evaluation, as the incidence of certain diseases can be quite unpredictable.

Not all animals, which were excluded on the basis of the clinical biochemical/ pathological evaluation, showed pharmacokinetic and pharmacodynamic parameters deviating from those measured in healthy animals. Arbitrarily, a parameter was considered deviant, if the difference from the lowest or the highest value of the parameter concerned in the healthy animals in the age group was more than 25%. It is difficult, however, to conclude what kind of diseases do have an influence on pharmacokinetics and pharmacodynamics, because of the variety in the kind and degree of diseases and because of multiple pathology in many animals. Moreover, concerning the pharmacodynamics three different effects (anaesthetic effect, EEG effect and anticonvulsant effect) were used and each effect was measured for two different model compounds. It is possible that a disease does influence the extent of one effect without affecting that of another one, or the extent of an effect for one model compound but not for the other model compound. This was observed in rats with liver abnormalities, where the disease influenced the pharmacodynamics of midazolam-induced EEG effects (an increase in the midazolam plasma concentration needed to reach half the maximum effect), but not the pharmacodynamics of heptabarbital-induced EEG effects. The influence of a disease on the same parameter even seems not always to be consistent: the pharmacokinetics and pharmacodynamics of oxazepam were influenced by a granular cell tumour in one animal (increase in elimination half-life and shift in concentration - anticonvulsant effect relationship to lower effect values at comparable concentrations), but not in another one.

Comparing all data (without considering the exact kind and degree of a disease and the kind of pharmacological effect parameter), about 50% of the pathology of the heart, the lungs and the kidney gave rise to changes in pharmacokinetics and/or pharmacodynamics (it should be emphasized that pathology of heart and lungs was mostly seen in combination with other kinds of pathology in our rats). Pathology of the liver gave rise to changes in pharmacokinetics and/or pharmacodynamics in about 70% of the cases and pathology of the brain in about 40%.

In chapter 6 it is discussed that measuring only clinical biochemical indices

in order to evaluate the health status of the rats in studies on ageing may be of limited value, because rats, which showed important abnormalities in the post-mortem tissue examination did not always show abnormal values for the clinical biochemical indices. Overviewing all diseased rats in all investigations in this thesis, it appears that not all individual rats with pathological abnormalities show abnormal values for the clinical biochemical indices (about 35% of the rats with pathological abnormalities did not show deviating values for the clinical biochemical indices, 30% showed one and 35% more than one deviation). In table 2 the mean values of the clinical biochemical indices of the diseased and healthy animals in the same age group (considering all rats included in this thesis) are given for the age groups with more than one diseased animal. It appears that the mean values in the healthy and diseased rats of the same age only showed a significant increase for blood urea nitrogen and a decrease for urine osmolality in the age group 28-31 months and an increase for plasma albumin and a decrease for blood glucose in the age group of 34-37 months. This emphasizes the necessity of a post-mortem tissue examination to determine the health status of the rats. This presents an important limitation of longitudinal studies, in which each animal is investigated several times throughout its life span, because one can only once perform a post-mortem tissue examination.
Concluding it can be stated that a prerequisite for application of male BN/BiRij rats as an animal model of ageing to study ageing *per se* is the exclusion of diseased animals on the basis of an extensive post-mortem tissue examination.

2. Cross-sectional vs. longitudinal study design

In the general introduction (chapter 2) the advantages and disadvantages of the two main study designs in gerontological investigations have been discussed (see table 3 in this chapter for an overview) (Rowe, 1977). For all studies described in this thesis, except one, a cross-sectional design was chosen. For application of the longitudinal design (in which each animal is subjected to investigations several times throughout its lifetime) in the pharmacodynamic studies in this thesis, it is in principle necessary for cannulas and electrodes to remain patent over the lifetime of the animal, which is of course not possible. Moreover, a post-mortem tissue examination shortly after the investigations, which appeared to be necessary to differentiate between ageing *per se* and age-related pathology (chapter 6), is

Table 2. *Clinical biochemical indices in different age groups of healthy and diseased male BN/BiRij rats, respectively.*

age (month) healthy or diseased rats	12-15 healthy	12-15 diseased	21-26 healthy	21-26 diseased	28-31 healthy	28-31 diseased	34-37 healthy	34-37 diseased
number of animals	60	3	54	4	47	9	26	9
body weight (g)	374 ± 5	380 ± 18	409 ± 4	443 ± 15	401 ± 5	391 ± 12	370 ± 10	384 ± 17
liver weight (g)	10.1 ± 0.2 (n=59)	9.7 ± 0.1	11.0 ± 0.2 (n=49)	13.0 ± 0.9	11.8 ± 0.4 (n=46)	11.0 ± 0.9	11.9 ± 0.5 (n=25)	12.7 ± 0.7
liver wt/ body wt (%)	2.71 ± 0.04 (n=59)	2.55 ± 0.10	2.73 ± 0.05 (n=49)	2.94 ± 0.12	2.98 ± 0.08 (n=46)	2.86 ± 0.30	3.19 ± 0.13 (n=25)	3.35 ± 0.20
blood aspartate aminotransferase (I.U./l)	86.3 ± 2.8	69.7 ± 2.8	79.6 ± 3.1	120 ± 34	78.9 ± 2.3	83.7 ± 5.3	110 ± 31	99.5 ± 5.5
blood alanine aminotransferase (I.U./l)	41.0 ± 1.2	40.1 ± 2.3	34.9 ± 0.8	35.0 ± 4.2	36.0 ± 1.0	37.3 ± 1.4	48 ± 14	31.0 ± 2.7
plasma albumin (mg/ml)	33.5 ± 0.9 (n=59)	39.9 / 28.7[A]	34.7 ± 1.5	30.9 ± 3.5	31.0 ± 0.7	29.1 ± 2.4	27.8 ± 1.8	35.7 ± 3.1[a]
plasma total protein (mg/ml)	87.4 ± 1.0	87.8 ± 1.6	87.0 ± 1.2	81.8 ± 2.5	87.9 ± 1.2	89.2 ± 4.4	84.4 ± 1.6	90.4 ± 5.0
blood glucose (mmol/l)	5.3 ± 0.1	4.9 ± 0.2	4.9 ± 0.2	5.6 ± 0.4	5.0 ± 0.1	5.3 ± 0.4	4.9 ± 0.2	3.9 ± 0.4[a]
blood urea nitrogen (mmol/l)	8.2 ± 0.1	8.0 ± 0.7	7.6 ± 0.2	8.4 ± 0.5	7.7 ± 0.2	8.8 ± 0.6[a]	8.0 ± 0.3	8.8 ± 0.5
creatinine clearance (ml/h.kg)	203 ± 8	154 ± 12	195 ± 9 (n=53)	204 ± 54	173 ± 9	173 ± 19	162 ± 9	139 ± 22
urine production (ml/24 hrs)	9.4 ± 0.4	6.4 ± 1.0	10.6 ± 0.5	8.4 ± 0.6	12.6 ± 0.7	14.7 ± 2.1	13.5 ± 1.2	15.8 ± 2.0
osmolality (mOsm/l)	1656 ± 37	1410 ± 160	1433 ± 39	1324 ± 96	1239 ± 32	951 ± 95[a]	1139 ± 48	957 ± 98

Results are presented as mean ± SEM

[A] individual values

[a] significantly different from value of the healthy animals of the same age, $p < 0.05$

not possible in the common longitudinal design.
A longitudinal design would offer important advantages in case of the existence of an extensive interindividual variability in the parameter under investigation. In the study on age-related changes in the pharmacodynamics of the midazolam-induced EEG effect (chapter 8) a large interindividual variability was observed, which limits the 'power' to detect age-related changes in the cross-sectional design.
In order to investigate, whether the results of our cross-sectional studies on the influence of ageing on pharmacodynamics are due to ageing or to differences between cohorts, the cross-sectional study on ageing and the pharmacodynamics of the anaesthetic effect of phenobarbital, described in chapter 4, was also performed in a 'pseudo'-longitudinal design. In this design, one group of animals, born within a period of two weeks, was reserved for the study and at five different ages one subgroup was investigated. At the start of the study, the animals were already assigned to one of the five subgroups. This 'pseudo'-longitudinal design does not require cannulas to remain patent during the whole lifetime of each animal. Moreover, because each animal was investigated only once, it was possible to perform a post-mortem tissue examination shortly after the investigations at regular lifetime intervals. In addition, 'carry-over' effects could not occur. The results of the 'pseudo'-longitudinal study confirmed those of the previous cross-

Table 3. *Advantages of longitudinal and cross-sectional study design in studies on age-related changes in pharmacodynamics.*

longitudinal design	cross-sectional design
- reduction of variability and increased power to detect statistically significant changes	- no 'carry-over' effects
	- cannula and electrode patency
- no misinterpretation of results because of selection of biologically superior survivors	- possibility of post-mortem tissue examination directly after the investigations
- only animals of one cohort included (no genetic differences)	- measurements are not subject to variability due to seasonal variability or different investigators

sectional study, thereby justifying the use of a cross-sectional set up in these kind of experiments. In addition, the 'pseudo'-longitudinal design offers a good alternative design to study age-related pharmacodynamic changes, by overcoming disadvantages of both a cross-sectional and a longitudinal design.

3. Age range to be included

In humans, the most important age-related changes in pharmacokinetics and pharmacodynamics often appear above the age of 60 years (Christensen and Andreasen, 1978; Bell et al., 1987; Roberts and Turner, 1988). In our rats, we observed both gradual changes during ageing, already starting at young age, and no important changes in young and mature rats, but more extensive changes in senescent rats. For the anaesthetic effect of phenobarbital (chapters 4 and 5) and for the anticonvulsant effect of sodium valproate (chapter 10) we found a gradual increase in brain sensitivity during ageing. The most important change in brain sensitivity for the anaesthetic effect of heptabarbital occurred above the age of 24 months (chapter 6), and for the EEG effects of midazolam and the anticonvulsant effect of oxazepam the most extensive changes were observed above the age of 30 months (chapters 8 and 9, respectively). These results emphasize the importance of including senescent rats (ages between 50% and 10% survival) and of investigating more than two age groups (in order to be able to study at what age the changes start) in pharmacological/ gerontological studies.

Pharmacological aspects:

1. Pharmacokinetics

In the investigations described in this thesis, data were gathered on the age-related changes in the pharmacokinetics of phenobarbital, heptabarbital, midazolam and oxazepam. For sodium valproate only the protein binding and the distribution between plasma, brain tissue and cerebrospinal fluid were studied.

For phenobarbital, heptabarbital, midazolam and oxazepam no consistent significant age-related changes in volume of distribution were observed (chapters 4, 5, 6, 8 and 9). The protein binding for all model compounds

investigated remained constant with increasing age. The distribution into the brain was assessed for phenobarbital, heptabarbital and sodium valproate by measuring concentrations in plasma, cerebrospinal fluid and brain tissue. The distribution of heptabarbital and sodium valproate over these three compartments did not show changes with increasing age (chapters 6 and 10), whereas for phenobarbital a small decrease in the amount of drug in the brain and cerebrospinal fluid relative to the plasma concentration was found in the 31- and 34-month-old rats (chapters 4 and 5).

The influence of ageing on renal clearance was investigated for phenobarbital and a decrease between the ages of 4 and 26 months was observed. In rats, the main route of elimination of phenobarbital, heptabarbital, midazolam and oxazepam is metabolism by the liver, primarily by oxidation reactions (phase I metabolism). Phenobarbital, midazolam and oxazepam are hydroxylated (Levin, 1986 and chapter 4; Woo et al., 1981; Sisenwine and Tio, 1986) and the reported metabolites of heptabarbital are also all formed by oxidation reactions (Gilbert et al., 1974; Vermeulen, 1980; Heeremans et al., in press; Stijnen et al., submitted). The total clearance showed an age-related tendency to decrease for phenobarbital and midazolam, resulting in a tendency to an increased elimination half-life (chapters 4, 5 and 8). For heptabarbital only the 24-month-old rats showed a relatively low clearance, resulting in a high mean residence time (chapter 6) and for oxazepam no age-related changes were found (chapter 9).

Summarizing, no major age-related changes in the pharmacokinetics of our model compounds were observed. This is in agreement with the findings of Groen (1991), who studied the pharmacokinetics of antipyrine, hexobarbital and theophylline in ageing BN/BiRij rats. He did not observe age-related changes in volumes of distribution of these compounds and a decrease in clearance only for theophylline. As will become clear from the following paragraph, age-related changes in the pharmacodynamics of our model compounds were more pronounced than those observed in the pharmacokinetics.

2. The influence of ageing on the brain sensitivity to the different drugs

2.1. Anaesthetic effect

The studies on the influence of age on the pharmacodynamics of the anaesthetic effect of the two barbiturates phenobarbital and heptabarbital showed a gradual increase in brain sensitivity to the anaesthetic effect from the age of 4 months to the age of 36 months for phenobarbital (chapters 4 and 5) and a constant sensitivity until the age of 24 months and an increase thereafter for heptabarbital (chapter 6). These findings show an age-related increase in brain sensitivity to the anaesthetic effect of barbiturates. These results, together with results on the EEG effect and the anticonvulsant effect, will be compared to results in humans in paragraph 5.

2.2. EEG effects

The pharmacodynamics of the heptabarbital-induced EEG effects appeared not to be influenced by ageing (chapter 7), whereas the brain sensitivity for the EEG effects of midazolam showed a tendency to increase between the ages of 29 and 36 months (chapter 8). Due to the substantial interindividual variability this did not reach statistical significance. These results suggest that the brain sensitivity for the EEG effects of barbiturates and benzodiazepines is differentially affected by ageing.

For benzodiazepines, it was shown that the EEG effects reflect the interaction of these compounds with the GABA-benzodiazepine receptor complex (Mandema et al., 1991). We did not observe age-related differences in the benzodiazepine receptor binding characteristics in our rats (chapter 9). Literature data on the influence of ageing on the GABA-benzodiazepine receptor complex do not show consistent age-related changes (Calderini et al., 1981; De Blasi et al., 1982; Komiskey and MacFarlan, 1983; Concas et al., 1988; Ito et al., 1988; Barnhill et al., 1990) (for a review: see chapter 1). For barbiturates the relationship between the EEG effects and the pharmacological effects is not known. Both barbiturates and benzodiazepines can exert several different pharmacological effects (Goth, 1984). The pharmacological effect profiles of these two classes of drugs are in part overlapping. The differential effect of age on the pharmacodynamics of barbiturates and benzodiazepines may suggest that the EEG effects of these two classes of drugs reflect different components of their pharmacological effect profiles.

2.3. Anticonvulsant effect

For the anticonvulsant effect of oxazepam and sodium valproate an increased brain sensitivity was observed with increasing age (chapters 9 and 10). The most important increase appeared to occur between the ages of 31 and 35 months for oxazepam, whereas for sodium valproate a more gradual increase over the lifetime was found, with the most pronounced changes taking place above the age of 25 months. Interestingly, the findings for oxazepam showed that the increase in brain sensitivity may be caused by a decrease in the effectiveness of homeostatic mechanisms, because an observed tolerance and withdrawal phenomenon in younger rats disappeared in the 36-month-old rats (chapter 9). This may also be the case with sodium valproate. However, the experimental design we used (chapter 10) does not allow conclusions in this respect.

The fact, that with increasing age an increased brain sensitivity for the anticonvulsant effect of two anticonvulsants with quite different mechanisms of action has been observed, indicates that a quite general mechanism may be involved. This may have major implications for the therapy of anticonvulsant drugs in general at high ages: a possible decreased dose requirement should be taken into account. Also for therapeutic drug monitoring a shift in the concentration vs. anticonvulsant effect relationships towards lower concentrations in the elderly compared to the young should be considered.

3. Comparison of the influence of ageing on the pharmacodynamics of the anaesthetic and the EEG effect of the same compound

Heptabarbital was included as a model drug in both a study on the influence of ageing on the pharmacodynamics of anaesthetic effects and a study on EEG effects and this allows therefore comparison of these two effects (chapters 6 and 7). Ageing did not appear to influence the pharmacodynamics of the EEG effect of this drug, whereas for the anaesthetic effect an increase in brain sensitivity between the ages of 24 and 30 months was observed. In studies in humans the pharmacodynamics of both the anaesthetic effect (using the threshold concentration at loss of eyelash reflex as pharmacodynamic parameter) and the EEG effects (using the spectral edge calculated by Fourier analysis as pharmacodynamic parameter) of thiopental did not show age-related changes (Christensen et al., 1981, 1982; Homer and

Stanski, 1985; Stanski and Maitre, 1990). Our results suggest that the EEG effect parameter we used may not be a good reflection of the anaesthetic effect of heptabarbital in BN/BiRij rats. Comparing the EEG effect and the anaesthetic effect, it is imaginable that more complex control mechanisms are involved in the pharmacodynamics of the anaesthetic effect. Shock already stated in 1961: 'Thus far, the largest age decrements that have been observed in intracellular processes are no greater than 15 per cent in contrast to decrements of 40 to 60 per cent in total organ performances.' He suggested that the impaired performance in the ageing adult is related to the breakdown in neural and endocrine regulatory mechanisms. Translating this statement to our results may indeed suggest the involvement of more complex regulatory mechanisms in the pharmacodynamics of the anaesthetic effect as compared to the EEG effect. This may explain why ageing does influence the pharmacodynamics of the anaesthetic effect of heptabarbital without influencing the pharmacodynamics of the EEG effect, as measured in our experiments.

4. At which pharmacodynamic level do the most important age-related changes take place?

Age-related changes in pharmacodynamics can be caused by changes in drug receptor interaction, post-receptor effectuation mechanisms and homeostatic mechanisms (Danhof, 1989). The investigations in this thesis mainly concern *in vivo* pharmacodynamic studies, in order to include all regulatory mechanisms in the investigations. In one *in vitro* study, the benzodiazepine receptor binding characteristics in whole brain homogenates from rats of different ages were determined and no age-related changes were observed (chapter 9). In chapter 1 (general introduction), literature data on drug-receptor interaction and post-receptor effectuation mechanisms for benzodiazepines and barbiturates were reviewed. These data do not give consistent evidence for age-related changes in these processes. The comparison of our results on the influence of ageing on the pharmacodynamics of the anaesthetic and EEG effect of barbiturates (as discussed above) suggest that the involvement of control mechanisms may lead to an age-related increased sensitivity to these effects, due to impairment of these control mechanisms. Moreover, the observed disappearance of tolerance and withdrawal syndrome to the anticonvulsant effect of oxazepam in senescent rats suggests that the impairment of

homeostatic mechanisms may be an important cause for the increased brain sensitivity to the anticonvulsant effect of this drug in the senescent rats (chapter 9).
Concluding, it can be stated that the results of our investigations, combined with literature data on the influence of age on drug receptor interaction and post-receptor events, suggest that the impairment of homeostatic mechanisms may be a major cause of age-related changes in pharmacodynamics of drugs, acting on the central nervous system, in rats. This emphasizes the importance of *in vivo* pharmacodynamic studies.

5. Consistency of results in BN/BiRij rats with regard to the situation in humans

Whether the ageing BN/BiRij rats are a suitable animal model for age-related pharmacokinetic and pharmacodynamic changes in the human situation can be evaluated by comparing findings in the rats with available data in man. The data which are available both in our rats and in man concern mainly data on pharmacokinetics. The data on phenobarbital pharmacokinetics during ageing in BN/BiRij rats agree qualitatively with those in humans on elimination half-life (a tendency to increase during ageing was observed), but disagree on renal clearance (in rats a decrease until the age of 26 months was observed in renal clearance and in fraction of the dose excreted unchanged in urine, with no change in fraction excreted unchanged in urine in humans) (chapter 4, Traeger et al., 1974). For midazolam the data on steady state volume of distribution and volume of the central compartment during ageing agree (no change during ageing), whereas the data on total clearance and elimination half-life disagree (in rats no age-related changes, in humans an age-related decrease in clearance and increase in elimination half-life were observed) (chapter 8; Greenblatt et al., 1984). This discrepancy may be caused by the different influence of age on liver weight between rats and humans (age-related decrease in humans is not observed in our rats) (Thompson and Williams, 1965). For oxazepam no age-related changes in pharmacokinetics were found in both humans and BN/BiRij rats (chapter 9; Murray et al., 1981). The protein binding for all model compounds studied appeared to be unaffected by age in our rats, which is in agreement with the data in humans for phenobarbital, midazolam and oxazepam, but not for sodium valproate (in humans an age-related decrease was observed) (chapters 4, 5, 6, 8, 9 and 10; Wallace and Verbeeck, 1987). For the majority

of these findings, it can be concluded that our data about age-related changes in pharmacokinetics correlate rather well with similar observations in humans.

More important in the context of this thesis, however, is the correlation between data on the influence of ageing on pharmacodynamics in our rats and in man. In this respect, it is important that the age-related decrease in anaesthetic phenobarbital dose requirement in our rats is qualitatively in agreement with the decreased anaesthetic thiopental dose requirement in humans (chapters 4 and 5; Christensen and Andreasen, 1978; Stanski and Maitre, 1990). The mechanism behind this change can be age-related changes in pharmacodynamics. However, in our rat studies we found an age-related decrease in anaesthetic threshold concentration of phenobarbital and heptabarbital, whereas the anaesthetic threshold concentration for thiopental in humans was independent of age (chapters 4, 5 and 6; Becker, 1978; Christensen et al., 1981, 1982). THe mechanism behind the age-related decrease in dose requirement appears therefore to be different in humans and rats. Our findings on the pharmacodynamics of heptabarbital-induced EEG effects not being influenced by ageing agree with findings on thiopental-induced EEG effects in man (chapter 7; Stanski and Maitre, 1990). Moreover, the disappearance of the withdrawal phenomenon to the anticonvulsant effect of oxazepam at the age of 36 months in our rats agrees with the decreased frequency at which withdrawal syndrome is reported as an adverse effect of benzodiazepines in the elderly (chapter 9; Tanner et al., 1989).

Obviously, only a qualitative comparison of data in humans and rats is possible. Moreover, a good comparison of data in humans and our rats is difficult, because the study design of most pharmacodynamic studies in humans is different from the design that is used in our rat studies. In several pharmacodynamic studies in humans, pharmacokinetic complicating factors, i.e. the formation of (inter)active metabolites (Dingemanse et al., 1988; Danhof, 1989; Danhof et al., in press), and age-related changes in baseline effect levels have not been taken into account. Next to the extent of age-related changes, also the age of onset of these changes is an important item. An additional criterium in the comparison of results of studies in man and in rats could therefore be the age of onset of age-related differences. This can only be assessed if more than two age groups (young and old) are included in the investigations in both humans and rats.

In order to be able to conclude whether our animal model is suitable in

predicting age-related changes in pharmacodynamics in man, more data on human pharmacodynamics are required. In order to collect these data, quantitative pharmacodynamic studies in humans should be performed including more than two age groups and taking pharmacokinetic interfering factors into account. Application of pharmacokinetic-pharmacodynamic modelling concepts may be valuable in this respect.

Perspectives for future research

The investigations described in this thesis represent some of the few studies in which age-related changes in *in vivo* pharmacodynamics of drugs acting on the central nervous system are investigated in a very systematic way. Since no general conclusions can be drawn (yet) on the influence of ageing on the pharmacodynamics of these drugs, more drugs have to be studied. It is possible that no general conclusion can be drawn on the influence of ageing on the pharmacodynamics of drugs of the same class. In that case, the drugs have to be studied individually. In addition, if the pharmacological profile of a drug consists of more than one pharmacological effect (as is for example the case for benzodiazepines), the possible age-related changes of the pharmacodynamics of these effects should be studied individually. This is important, because ageing does not necessarily influence the pharmacodynamics of different effects exerted by the same drug in the same way (chapters 6 and 7). Moreover, these investigations should be extended to more classes of drugs acting on the central nervous system and other drugs frequently used in the elderly, because for many drugs influence of ageing on their pharmacodynamics is still a relatively unexplored research field. As the Committee on a National Research Agenda on Aging (1991) stated, 'more studies of the fundamental pharmacodynamics of various medications in older individuals would forward clinical care'. *In vivo* studies applying the pharmacokinetic-pharmacodynamic modelling concept may be valuable in this respect.

In the investigations described in this thesis, homeostatic mechanisms appeared to be of major importance for the age-related changes in pharmacodynamics of drugs acting on the central nervous system. The nature of these homeostatic mechanisms is not known. A similar conclusion was drawn from studies on the influence of ageing on the pharmacokinetics (Groen, 1991). It was concluded that the most important decline at high ages

is observed in the 'flexibility'/ reserve capacity of the body, because of the relatively large age-related decrease in the enzyme inducing effect of phenobarbital. For the pharmacodynamics of drug effects on the central nervous system both more extensive studies on homeostatic mechanisms, involved in the pharmacodynamics of these effects, and studies on the influence of ageing on these mechanisms are indicated.

Until now, not enough evidence is available on the predictive value of the applied animal model of ageing (male BN/BiRij rats of different ages between 3 and 37 months) for the situation in man, concerning the influence of age on the pharmacodynamics of drugs acting on the central nervous system. Therefore, studies are indicated in which the influence of age on the pharmacodynamics of these drugs is investigated in man a way comparable to the methods applied in our rat studies (using quantitative measures of the pharmacological effect, taking pharmacokinetic interfering factors into account, including more than two age groups, evaluating the health status of the subjects). It is interesting that the method to measure and evaluate EEG effects can also be applied in human studies (Mandema et al., submitted 1). Other techniques which can be applied in humans are positron emission tomography (PET) and single photon emission tomography (SPET). Ultimately these techniques should enable monitoring local drug concentrations and *in vivo* receptor occupancy (Mazière and Mazière, 1990), offering the possibility for more mechanistic research. Both the validation of animal models of ageing and pharmacodynamic research into the mechanisms behind age-related changes can be important aims for application of these new techniques.

The Committee on a National Research Agenda on Aging (1991) mentioned polypharmacy as a significant cause of morbidity and hospitalization among elderly patients, thereby emphasizing the importance of drug-drug interaction studies in an ageing population. Pharmacokinetic-pharmacodynamic modelling concepts may be very useful in this respect (Mandema et al., submitted 1 and 2).

Since next to age disease appeared to be a possible cause for pharmacodynamic changes in the elderly, studies on the influence of age-associated pathology on pharmacodynamics are necessary to be able to predict the dose requirement in individual elderly patients. Therefore the interaction between age and disease should be studied, especially for diseases that predominantly occur at higher ages. In addition, the

pharmacodynamics of drugs should be studied in the patient group, which will be the ultimate users of the drug. The application of population pharmacodynamics (Sheiner and Benet, 1985; Aarons et al., in press) may offer an interesting possibility to study both the interaction between age and pathology and the above mentioned drug-drug interactions in the elderly.

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References

Aarons, L., Mandema, J.W. and Danhof, M.: A population analysis of the pharmacokinetics and pharmacodynamics of midazolam in the rat. J. Pharmacokin. Biopharm., in press.

Barnhill, J.G., Greenblatt, D.J., Miller, L.G., Gaver, A., Hartmatz, J.S. and Shader, R.I.: Kinetic and dynamic components of increased benzodiazepine sensitivity in aging animals. J. Pharmacol. Exp. Ther. 253: 1153-1161, 1990.

Becker, K.E.: Plasma levels of thiopental necessary for anesthesia. Anesthesiol. 49: 192-196, 1978.

Bell, G.D., Spickett, G.P., Reeve, P.A., Mordon, A. and Logan, R.F.A.: Intravenous midazolam for upper gastrointestinal endoscopy: a study of 800 consecutive cases relating dose to age and sex of patient. Br. J. clin. Pharmacol. 23: 241-244, 1987.

Brody, J.A. and Schneider, E.L.: Diseases and disorders of aging: an hypothesis. J. Chron. Dis. 39: 871-876, 1986.

Burek, J.D.: Pathology of ageing rats. A morphological and experimental study of the age-associated lesions in aging BN/Bi, Wag/Rij and (WAG x BN) F_1 rats. CRC-Press Inc., West Palm Beach, Florida, 1978.

Calderini, G., Bonetti, A.C., Aldinio, A., Savioni, G., Di Perri, B., Biggio, G. and Toffano, G.: Functional interaction between benzodiazepine and GABA recognition sites in aged rats. Neurobiol. Aging 2: 309-313, 1981.

Christensen, J.H. and Andreasen, F.: Individual variation in response to thiopental. Acta Anaesthesiol. Scand. 22: 303-313, 1978.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Influence of age and sex on the

pharmacokinetics of thiopentone. Br. J. Anaesth. 53: 1189-1195, 1981.

Christensen, J.H., Andreasen, F. and Jansen, J.A.: Pharmacokinetics and pharmacodynamics of thiopentone. A comparison between young and elderly patients. Anaesthesia 37: 398-404, 1982.

Committee on a National Research Agenda on Aging: Extending life, enhancing life. E.T. Lonergan, ed., National Academy Press, Washington, D.C., 1991.

Concas, A., Pepitoni, S.J., Atsoggiu, T., Toffano, G. and Biggio, G.: Aging reduces the GABA dependent ^{36}Cl-flux in rat brain membrane vesicles. Life Sci. 43: 1761-1771, 1988.

Danhof, M.: Does variability in drug disposition explain (all) variability in drug effects? in: Topics in Pharmaceutical Sciences 1989, D.D. Breimer, K.K. Midha and D.J.A. Crommelin, eds., Amsterdam Medical Press, Noordwijk, The Netherlands, 1989, pp 573-586.

Danhof, M., Mandema, J.W. and Stijnen, A.M.: Pharmacokinetic complexities of pharmacodynamic studies *in vivo*. in: The *in vivo* study of drug action. Principles and applications of kinetic-dynamic modelling. C.J. van Boxtel, M. Danhof and N.H.G. Holford, eds., in press.

De Blasi, A., Cotecchia, S. and Mennini, T.: Selective changes of receptor binding in brain regions of aged rats. Life Sci. 31: 335-340, 1982.

Dingemanse, J., Danhof, M. and Breimer, D.D.: Pharmacokinetic-pharmacodynamic modelling of CNS drug effects: An overview. Pharmacol. Ther. 38: 1-52, 1988.

Gilbert, J.N.T., Millard, B.J., Powell, J.W. and Whalley, W.B.: Metabolism of heptabarbitone. J. Pharm. Pharmacol. 26: 123-125, 1974.

Goth, A.: Medical Pharmacology. 11th edition. S.E. Harshberger, ed., The C.V. Mosby Company, St. Louis, Toronto, 1984.

Greenblatt, D.J., Abernethy, D.R., Lockniskar, A., Harmatz, J.S., Limjuco, R.A. and Shader, R.I.: Effect of age, gender and obesity on midazolam kinetics. Anesthesiol. 61: 27-35, 1984.

Groen, C.: The influence of ageing on P450-mediated drug metabolism. Ph.D. Thesis. University of Leiden, 1991.

Heeremans, C.E.M., Stijnen, A.M., Van der Hoeven, W.M.A., Niessen, W.M.A., Danhof, M. and Van der Greef, J.: Thermospray liquid chromatography/tandem mass spectrometry for identification of a heptabarbital metabolite and sample work-up artefacts. J. Chromatogr. 554: 205-214, 1991.

Hollander, C.F., Delort, P. and Duprat, P.: Toxicology in the older age group. in: Progress in predictive toxicology. D.B. Clayson, I.C. Munro, P. Shubik, J.A. Swenberg, eds., Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam, 1990, pp 113-142.

Homer, T.D. and Stanski, D.R.: The effect of increasing age on thiopental disposition and anesthetic requirement. Anesthesiol. 62: 714-724, 1985.

Ito, Y., Ho, I.K. and Hoskins, B.: Cerebellar GABA and benzodiazepine receptor characteristics in young and aged mice. Brain. Res. Bull. 21: 251-255, 1988.

Levin, S.S., Vars, H.M., Schleyer, H. and Cooper, D.Y.: The metabolism and excretion of enzyme-inducing doses of phenobarbital by rats with bile fistulas. Xenobiotica 16: 213-224, 1986.

Mandema, J.W., Sansom, L.N., Dios-Vièitez, M.C., Hollander-Jansen, M. and Danhof, M.: Pharmacokinetic-pharmacodynamic modelling of the EEG effects of benzodiazepines. Correlation with receptor binding and anticonvulsant activity. J. Pharmacol. Exp. Ther. 257: 472-478, 1991.

Komiskey, H.L. and MacFarlan, M.F.: Aging: Effect on neuronal and non-neuronal benzodiazepine binding sites. Neurochem. Res. 8: 1135-1142, 1983.

Mandema, J.W., Tuk, B., van Steveninck, A.L., Breimer, D.D., Cohen, A.F. and Danhof, M.D.: Pharmacokinetic-pharmacodynamic modelling of the CNS effects of midazolam and its main metabolite α-hydroxy-midazolam in healthy volunteers. Submitted for publication (1).

Mandema, J.W., Kuck, M.T. and Danhof, M.: *in vivo* modelling of the pharmacodynamic interaction between benzodiazepines which differ in intrinsic efficacy. Submitted for publication (2).

Mazière, B. and Mazière, M.: Where have we got to with neuroreceptor mapping of the human brain? Eur. J. Nuc. Med. 16: 817-835, 1990.

Murray, T.G., Chiang, S.T., Koepke, H.H. and Walker, B.R.: Renal disease, age, and oxazepam kinetics. Clin. Pharmacol. Ther. 30: 805-809, 1981.

Roberts, J. and Turner, N.: Pharmacodynamic basis for altered drug action in the elderly. Clinics in Geriatr. Med. 4: 127-149, 1988.

Rowe, J.W.: Clinical research on aging: strategies and directions. New Engl. J. Med. 297: 1332-1336, 1977.

Sheiner, L.B. and Benet, L.Z.: Premarketing observational studies of population pharmacokinetics of new drugs. Clin. Pharmacol. Ther. 38: 481-487, 1985.

Shock, N.W.: Physiological aspects of aging in man. Ann. Rev. Physiol. 23: 97-122, 1961.

Sisenwine, S.F. and Tio, C.O.: The metabolic disposition of oxazepam in rats. Drug Metab. Disp. 14: 41-45, 1986.

Stanski, D.R. and Maitre, P.O.: Population pharmacokinetics and pharmacodynamics of thiopental: The effect of age revisited. Anesthesiol. 72: 412-422, 1990.

Stijnen, A.M., Heeremans, C.E.M., Van Bezooijen, C.F.A., Niessen, W.M.A. and Danhof, M.: Disposition of heptabarbital in the rat: identification of a new metabolite by tandem mass spectrometry. Submitted for publication.

Tanner, L.A., Baum, C., Prela, C.-M. and Kennedy, D.L.: Spontaneous adverse reaction reporting in the elderly for 1986. J. Geriatr. Drug Ther. 3: 31-55, 1989.

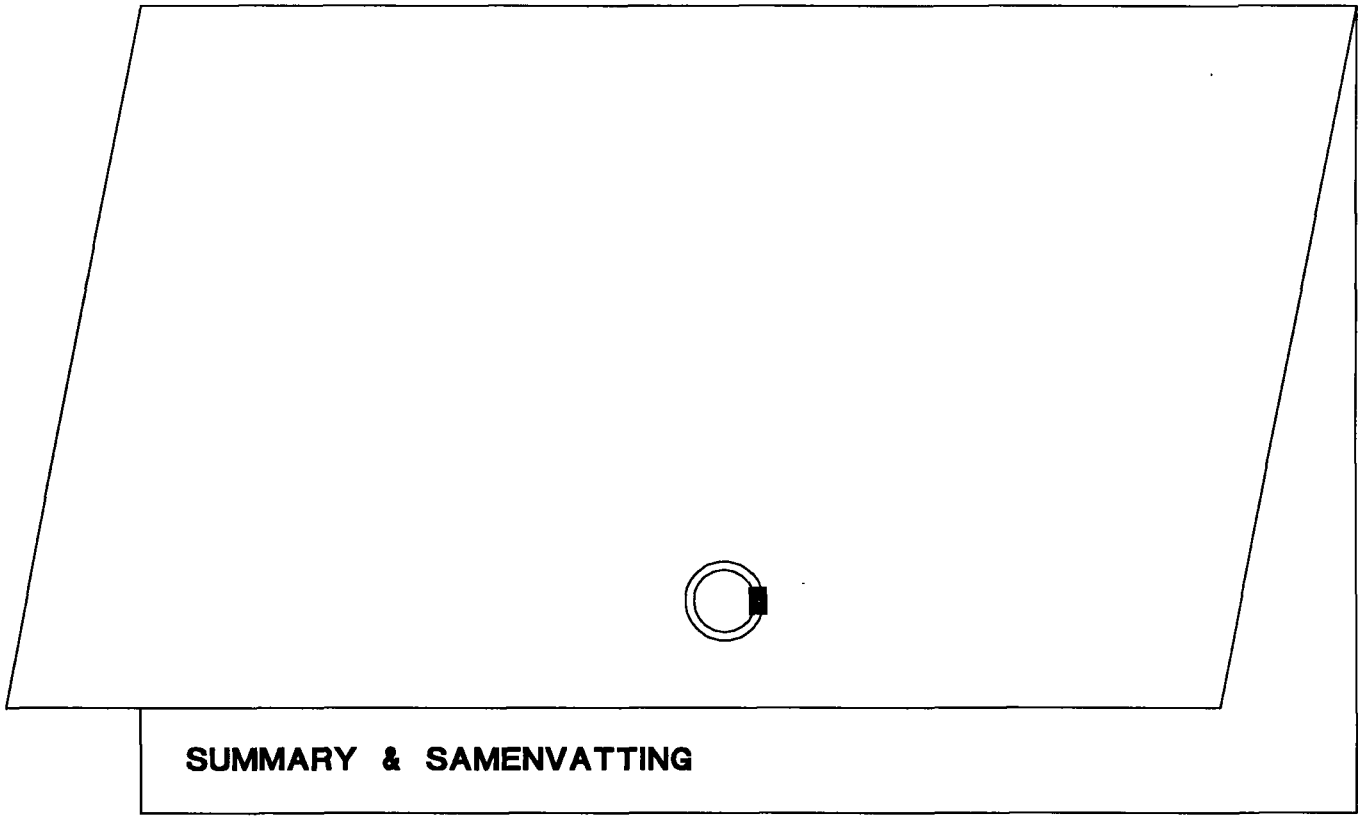
Thompson, E.N. and Williams, R.: Effect of age on liver function with particular reference to bromsulphalein excretion. Gut 6: 266-291, 1965.

Traeger, A., Kiesewetter, R. and Kunze, M.: Zur Pharmakokinetik von Phenobarbital bei Erwachsenen und Greisen. Dtsh. Ges. Wesen 29: 1040-1042, 1974.

Vermeulen, N.P.E.: The epoxide-diol pathway in the metabolism of hexobarbital and related barbiturates. Ph.D. Thesis, University of Leiden, 1980, pp 103-109.

Wallace, S.M. and Verbeeck, R.K.: Plasma protein binding of drugs in the elderly. Clin. Pharmacokin. 12: 41-72, 1987.

Woo, G.K., Williams, T.H., Kolis, S.J., Warinsky, D., Dasso, G.J. and Schwartz, M.A.: Biotransformation of [^{14}C]midazolam in the rat *in vitro* and *in vivo*. Xenobiotica 6: 373-384, 1981.



SUMMARY & SAMENVATTING

SUMMARY

Section I. General Introduction

It is generally acknowledged that both the absolute and relative number of elderly people in our society is still increasing. Since old age has its infirmities, more than 60% of the elderly people frequently uses one or more drugs. Cardiovascular drugs and drugs acting on the central nervous system are the most frequently prescribed. A major problem with drug therapy in the elderly is the relatively high incidence of adverse drug reactions. Next to this fact a decreased dose requirement compared to young subjects is observed in clinical geriatric practice. Two important causes for the increased incidence of adverse drug effects and the decreased dose requirement in the elderly can be age-related changes in pharmacokinetics and in pharmacodynamics of the drugs. Research has considerably improved our understanding of the influence of ageing on pharmacokinetics. Potential age-related changes in pharmacodynamics are still relatively unexplored, however.

In chapter 1 literature data on the influence of ageing on the pharmacodynamics of drugs acting on the central nervous system are reviewed, with special reference to barbiturates and benzodiazepines. Studies in man provided evidence for a decrease in dose requirement with increasing age. The results of several, but not all studies suggested also age-related changes in pharmacodynamics, consisting of an increase in brain sensitivity during ageing. *in vivo* animal studies also provided evidence for an increase in brain sensitivity to barbiturates and benzodiazepines during ageing, although also the results of these studies are not unambiguous. The interpretation of the results of the pharmacodynamic studies in humans and in rats is often difficult, due to changes in baseline effect during ageing and to possible age-related interfering pharmacokinetic changes, that are not taken into consideration, e.g. changes in distribution of the drug between plasma and site of action or in the formation of (inter)active metabolites. In addition, the changes observed during ageing in the animal studies may (in part) be due to age-associated pathology, because the health status of the animals has usually not been assessed.

The mechanism of possible age-related changes in pharmacodynamics is generally not known. An increase in brain sensitivity during ageing can be a result of age-related changes in drug-receptor interaction, post-receptor events (effectuation processes) or in homeostatic mechanisms. *In vitro* studies on drug-receptor interaction and post-receptor events did not give insight into the possible mechanism behind an increased brain sensitivity to barbiturates and benzodiazepines during ageing, because the findings of these studies are rather contradictory.
More extensive and rigorously designed *in vivo* pharmacodynamic studies, including investigations into age-related changes in baseline effect and pharmacokinetic changes, are needed to enlarge our insight into the effect of ageing on pharmacodynamics of drugs acting on the central nervous system. In this respect, studies in animal models of ageing may be particularly valuable. The aim of the studies described in this thesis was to investigate the influence of ageing on the *in vivo* pharmacodynamics of sedative and anticonvulsant drugs in rats, excluding interfering pharmacokinetic factors and the contribution of concomitant pathology. The gerontological and pharmacological aspects of the methodology of these studies are described in chapter 2. Important prerequisites for the rats used, are the availability of data on survival curves and on age-related pathology. Animals around the ages of 50 and 10% survival should be included. In addition, studying more than just two age groups ('young' and 'old') is important in order to be able to study changes over the whole age range (differences observed between young and old rats cannot be interpolated to intermediate ages). In our studies four to seven age groups of male BN/BiRij rats, aged between 3 and 37 months (about 100 and 15% survival), were used. The health status of all individual animals used was carefully assessed to be able to exclude diseased animals from the investigations and thereby to study ageing *per se*.

In gerontological research, in principle two different types of study design can be chosen: a cross-sectional and a longitudinal design. In the first design, different groups (cohorts) of rats of various ages are studied within a short period of time; in the second, serial measurements are obtained in one group of rats at specified ages during their lives. We applied the cross-sectional design in all studies but one; in that study a 'pseudo'-longitudinal design was applied. In that design, one groups of rats, born within a period of two weeks, was reserved for the study and at five different ages one subgroup was

studied. By applying the 'pseudo'-longitudinal design, disadvantages of both the cross-sectional and the common longitudinal design are overcome.
In our studies three different techniques to quantitate the pharmacological effect of the sedative and anticonvulsant drugs were used. The point of onset or offset of loss of righting reflex was used as a measure for the anaesthetic effect. The second technique applied drug-induced changes in the electroencephalogram (EEG) as a measure of the pharmacological effect. The individual concentration vs. EEG effect relationships could be characterized by the sigmoidal E_{max} model. The anticonvulsant effect was quantitated using a direct cortical stimulation method.
The results of pharmacodynamic studies can be influenced by several pharmacokinetic factors, like the distribution between the sampling site and the site of action, the formation of (inter)active metabolites and differences in the disposition of enantiomers of chiral compounds when administered as a racemate. These factors were taken into consideration in the studies described in this thesis.
The various investigations, described in this thesis, were introduced in chapter 3. Next to studying the influence of ageing on the pharmacodynamics of the anaesthetic effect of phenobarbital and heptabarbital, the EEG effects of heptabarbital and midazolam and the anticonvulsant effect of oxazepam and sodium valproate, attention was paid to the influence of age-associated pathology on changes in pharmacodynamics during ageing. In addition, the results of a cross-sectional study on the age-related changes in the pharmacodynamics of phenobarbital were compared to those obtained in a comparable study with a 'pseudo'-longitudinal design.

Section II. Anaesthetic effects

A cross-sectional study on the influence of ageing on the pharmacokinetics (following a bolus dose of 25 mg/kg) and pharmacodynamics (during infusion at a rate of 3 mg/min until onset of loss of righting reflex) of phenobarbital showed no important changes in pharmacokinetics during ageing and a decrease of about 30% between the ages of 4 and 36 months in both the phenobarbital threshold dose and cerebrospinal fluid concentration for the onset of loss of righting reflex (chapter 4). Since only minor changes in the metabolite profile and the distribution of phenobarbital between plasma (total and free), cerebrospinal fluid and brain tissue were observed, the age-related

decrease in threshold cerebrospinal fluid concentration can be attributed to an increase in brain sensitivity to phenobarbital during ageing. This age-related increase in brain sensitivity appeared to be the major cause for the decrease in dose requirement. Renal impairment has been reported to influence the sensitivity of the brain to phenobarbital, but in our study the increase in brain sensitivity was not correlated to parameters for kidney functioning.

Observed changes in studies performed with a cross-sectional design can, in principle, be due to cohort differences. Therefore the pharmacokinetics and pharmacodynamics of phenobarbital were also investigated in a comparable study with a 'pseudo'-longitudinal design (as described above). The results of this study confirmed those of the cross-sectional study, thereby excluding the contribution of cohort differences (chapter 5). In addition, in the 'pseudo'-longitudinal design several disadvantages of the cross-sectional and the common longitudinal design are overcome. Therefore it appears to be an interesting new approach.

In a study on the influence of ageing on the pharmacokinetic-pharmacodynamic relationship of heptabarbital (following intravenous administration of a heptabarbital bolus dose of 85 mg/kg), a relatively high percentage of the animals appeared to show severe pathology, as assessed by an independent pathologist (chapter 6). Therefore, the results were evaluated both including and excluding the diseased animals. In the group as a whole, an increase of about 110% in the duration of the anaesthetic effect was observed between the ages of 7 and 31 months, mainly due to a decrease in clearance with no apparent changes in pharmacodynamics. After excluding the diseased animals, an entirely different situation was observed: no major age-related pharmacokinetic changes but an increase in brain sensitivity between the ages of 24 and 31 months was found (reflected in a 30% decrease of the threshold heptabarbital concentration in cerebrospinal fluid). Comparison of the results obtained in five healthy and five diseased 31-month-old rats showed again a lower clearance and a tendency to a lower brain sensitivity in the diseased animals. It must be concluded therefore that concurrent disease can have important implications for the interpretation of the results, thereby emphasizing the importance of the assessment of the health status of the animals in this kind of studies. The assessment of the health status of the rats was performed on the basis of a post-mortem tissue evaluation and measurement of clinical biochemical indices. Not all animals

which showed severe pathology could be recognized by deviating clinical biochemical indices. This emphasizes the importance of including a post-morten tissue examination in the pathological evaluation.

Section III. EEG effects

Next to the study on the anaesthetic effect of heptabarbital, this barbiturate was studied on the basis of EEG changes (chapter 7). Heptabarbital was infused at a rate of 11 mg/kg.min until isoelectric periods in the EEG of 5 seconds duration. Utilizing the total amplitude as a pharmacodynamic measure, a monophasic effect (decrease) was observed in the frequency range of 2.5-30 Hz and a biphasic effect (increase followed by decrease) in the frequency range of 0.5-2.5 Hz. In both frequency ranges no age-related changes were found in the concentration vs. EEG effect relationships. These findings are contradictory to the increase in brain sensitivity to the anaesthetic effect of heptabarbital between the ages of 24 and 31 months (vide supra). In our rats, barbiturate-induced EEG changes may therefore not have a predictive value for age-related changes in sensitivity to the anaesthetic effects of barbiturates. Ageing is known to be associated with decreases in performance of control mechanisms. Since it is possible that more complex control mechanisms are involved in the pharmacodynamics of the anaesthetic effect, this may explain the difference in the influence of ageing on the pharmacodynamics of the anaesthetic and the EEG effect.
In chapter 8 a study on the influence of ageing on the pharmacokinetic-EEG effect relationship of midazolam (following an intravenous dose of 2.5 mg/kg) is described. The total amplitude in the frequency range of 11.5-30 Hz was used as a pharmacodynamic measure. No age-related changes in pharmacokinetics were observed. Concerning the pharmacodynamics, a tendency towards a decrease (of about 50%) in the midazolam concentration at half the maximum effect was observed in the 36-month-old rats, suggestive for an increased brain sensitivity. However, it appeared that also factors other than age contribute to interindividual variability in pharmacodynamics as well, because a substantial variability was observed within certain age groups.

Section IV. Anticonvulsant effects

The pharmacodynamics of the anticonvulsant effect of oxazepam (following an intravenous bolus dose of 12 mg/kg) showed an interesting phenomenon in young BN/BiRij rats: an anticonvulsant effect followed by a proconvulsant effect was observed in the pharmacological effect vs. time profile (chapter 9). This finding is suggestive for the occurrence of acute tolerance and/or withdrawal phenomenon. With increasing age the proconvulsant effect disappeared, resulting in a monophasic effect profile (anticonvulsant effect only) at the age of 35 months with significantly higher anticonvulsant effect intensity immediately after administration (about 100% higher than the effect intensity achieved in the 4-month-old animals). In the pharmacokinetics no age-related changes were observed.

In the *in vitro* benzodiazepine receptor binding characteristics no age-related changes were observed in both receptor affinity and density. Available literature data on post-receptor events do not indicate conclusive age-related changes. Therefore, the observed change in the pharmacodynamics of the anticonvulsant effect in the 35-month-old rats may be explained by the disappearance of the tolerance/withdrawal phenomenon. This is compatible with the generally accepted impairment of homeostatic control mechanisms in the elderly.

The pharmacodynamics of the anticonvulsant effect of sodium valproate were assessed in different age groups during intravenous infusion of the drug at a rate of 5.5 mg/kg.min (until the anticonvulsant effect intensity had reached the maximum technical attainable level or, when this did not take place within three hours after the start of the infusion, until a total infusion time of three hours). A parallel shift in the concentration vs. anticonvulsant effect relationship towards lower concentrations was observed with increasing age (the concentrations needed to achieve fixed effect intensity showed a decrease of about 40% between the ages of 3 and 37 months). This is suggestive for an age-related increase in sensitivity to the anticonvulsant effect of sodium valproate in BN/BiRij rats.

Section V. Conclusions and perspectives

In chapter 11 the general gerontological and pharmacological aspects of all studies are discussed. Concerning age-associated pathology, it is not possible to conclude in general what kind of diseases have an influence on pharmacokinetics and pharmacodynamics because of the variety in the kind and degree of diseases and because of multiple pathology in several animals. It appeared to be important to include all relevant organs and tissues in the pathological evaluation of every separate study, because of the fact that the incidence of certain diseases can vary extensively between different cohorts of rats. The conclusion in chapter 6, that a post-mortem tissue examination has to be a part of the pathological evaluation, was confirmed by results obtained in other studies in this thesis.

As was already mentioned as a general 'rule' in chapter 2, it also appears from our experiments that inclusion of age groups between 50 and 10% survival in the investigations is important, because the most pronounced changes often were observed in the senescent rats. Moreover, since the onset of age-related changes is not always observed at the same age, it is also important to include more than only two age groups.

No general conclusion can be drawn yet on the influence of ageing on the pharmacodynamics of sedative and anticonvulsant drugs. We observed an increase in brain sensitivity to the anaesthetic effect of barbiturates and to the anticonvulsant effect of oxazepam and sodium valproate. For oxazepam this increase seems to be mainly due to an age-related impairment of homeostatic control mechanisms. For sodium valproate the study design used does not allow conclusions in this respect. The pharmacodynamics of the EEG effect of heptabarbital did not show age-related changes, whereas for the EEG effect of midazolam a tendency to an increased brain sensitivity was observed. More studies are indicated with individual drugs, applying the pharmacokinetic-pharmcodynamic modelling concept. The results of our studies, combined with literature data on the influence of ageing on drug receptor interaction and post-receptor events, suggests that the impairment of homeostatic mechanisms may be a major cause for age-related increase in brain sensitivity to sedative and anticonvulsant drugs. This emphasizes the importance of pharmacodynamic investigations *in vivo*.

A challenge for future research is the assessment of the predictive value of the animal experiments for the situation in humans. In this respect, more

comparable *in vivo* pharmacodynamic studies applying the pharmacokinetic-pharmacodynamic modelling concept are indicated in humans and rats. EEG effect measurements may be an important technique, because EEG effects can be measured in both humans and rats in a comparable way.
As perspectives for future research, studies on influence of the interaction of age and age-associated disease are mentioned. Also studies on drug-drug interaction in the elderly are indicated, because of the often encountered polypharmacy in elderly patients. For these two kinds of studies, population pharmacodynamics may be an interesting tool. In addition, the newly developed techniques positron emission tomography (PET) and single photon emission tomography (SPET) may be applied to study the mechanism behind age-related pharmacodynamic changes in humans.

DE INVLOED VAN VEROUDERING OP DE MATE VAN WERKING VAN NARCOSEMIDDELEN EN GENEESMIDDELEN TEGEN EPILEPSIE IN DE RAT

UITGEBREIDE POPULAIR-WETENSCHAPPELIJKE SAMENVATTING

Deel I. Algemene inleiding

I.1. Achtergrond

Het is genoegzaam bekend, dat de wereldbevolking steeds ouder wordt. Omdat volgens het spreekwoord ouderdom met gebreken komt, stappen ouderen vaker over de drempel bij de apotheek. Ruim een kwart van het geld dat wordt uitgegeven aan geneesmiddelen komt voor rekening van 65-plussers. De geneesmiddelen die ouderen het meest frequent gebruiken zijn geneesmiddelen met een werking op het hart en de bloedvaten en geneesmiddelen die een werking hebben op de hersenen, bijv. slaapmiddelen. Vooral de zgn. benzodiazepines worden veelvuldig voorgeschreven. Dit zijn geneesmiddelen die o.a. worden toegepast bij narcose, als bescherming tegen epileptische aanvallen of als kalmerend middel (bijv. Valium®). Helaas verloopt de geneesmiddeltherapie bij de oudere patiënt niet altijd zonder problemen. Ouderen blijken vaker last te hebben van ongewenste bijwerkingen (bijv. duizeligheid) dan jongere patiënten. Bovendien blijkt regelmatig dat ze, vergeleken met jongeren, aan een lagere dosering genoeg hebben (een hálf slaaptabletje is bijv. al genoeg), hun dosisbehoefte is lager. De oorzaak voor deze bevindingen bij bejaarden kunnen liggen in leeftijdsafhankelijke veranderingen in één van de twee (of beide) volgende groepen processen die zich in het lichaam afspelen: de zgn. farmacokinetische processen (kortweg farmacokinetiek) en de farmacodynamische processen (kortweg farmacodynamiek). Farmacokinetiek houdt zich bezig met de vraag 'Wat doet het lichaam met het geneesmiddel?', farmacodynamiek met 'Wat doet het geneesmiddel met het lichaam?'. De

farmacokinetiek en de farmacodynamiek samen bepalen de dosisbehoefte en ook hoe sterk de bijwerkingen zijn. De eerstgenoemde processen omvatten de lotgevallen van een geneesmiddel in het lichaam. Wanneer iemand een geneesmiddel slikt komt het (meestal) via het maagdarmkanaal in het bloed terecht. Via het bloed wordt het geneesmiddel door het hele lichaam getransporteerd en door verschillende organen en weefsels opgenomen. Aangezien de weefselverdeling tijdens veroudering verandert (de hoeveelheid vetweefsel neemt bijv. toe en de hoeveelheid spierweefsel af), is de hoeveelheid geneesmiddel die in de verschillende weefsels wordt opgenomen bij ouderen verschillend van die bij jongeren. Het geneesmiddel wordt o.a. door de lever en de nieren opgenomen. Een geneesmiddel, dat goed in water oplost, wordt via de nieren met de urine uit het lichaam verwijderd. Andere geneesmiddelen worden eerst door de lever afgebroken (door stofwisselingsprocessen) en daarna uit het lichaam verwijderd. De werkzaamheid van de lever en de nieren kan achteruit gaan bij het ouder worden, waardoor het geneesmiddel langzamer uit het lichaam wordt verwijderd. Daardoor blijft er meer geneesmiddel in het bloed aanwezig, dat beschikbaar is om zijn werking uit te oefenen. Deze farmacokinetische processen zijn voor veel geneesmiddelen al uitgebreid onderzocht bij ouderen.

De tweede groep processen, die van belang kunnen zijn, zijn de farmacodynamische processen. Dit zijn de processen die door het geneesmiddel in gang worden gezet om uiteindelijk de werking van het geneesmiddel teweeg te brengen. Als voorbeeld voor deze processen kunnen de processen worden genoemd die de werking tot stand brengen van de geneesmiddelen die wij zelf hebben onderzocht. Deze werken alle op de hersenen. Nadat zo'n geneesmiddel via het bloed in de hersenen is opgenomen, bindt het eerst aan een specifiek eiwit, een zgn. receptor (het geneesmiddel past op deze receptor als een sleutel in een slot). Door deze binding wordt een serie opeenvolgende chemische processen in gang gezet, die uiteindelijk tot het effekt van het geneesmiddel leiden, zoals bijv. slaap of bescherming tegen een epileptische aanval. Bij het tot stand komen van de werking van het geneesmiddel kunnen ook regelmechanismen in het lichaam een rol spelen. Voorbeelden van regelmechanismen zijn die welke de lichaamstemperatuur of de bloeddruk op peil houden. Al deze processen samen (van binding aan het eiwit tot de regelmechanismen) noemen we de farmacodynamiek van een geneesmiddel. De farmacodynamische processen

zijn moeilijker te bestuderen dan de farmacokinetische en daarom is er nog niet zo veel bekend over veranderingen in die processen bij het ouder worden.

I.2. Doel van het onderzoek en stand van zaken vóór het onderzoek

Het doel van ons onderzoek was het bestuderen van de invloed van veroudering op de farmacodynamische processen, die een rol spelen bij de werking van narcosemiddelen (sedativa) en geneesmiddelen die beschermen tegen epileptische aanvallen (anticonvulsiva). Omdat farmacokinetische en farmacodynamische processen elkaar kunnen beïnvloeden, moet de farmacokinetiek ook worden onderzocht om de farmacodynamiek eenduidig te kunnen bestuderen. Dit geldt bijv. voor de afbraak van geneesmiddelen in de lever (stofwisseling) omdat bij deze afbraak stoffen gevormd kunnen worden die zelf ook weer een werking hebben. Het is dan belangrijk om te onderzoeken of een gemeten werking wordt teweeggebracht door het geneesmiddel zelf of door deze afbraakprodukten. Bij experimenten over leeftijdsgerelateerde veranderingen is het bovendien van belang om de invloed van de leeftijd te scheiden van de invloed van ziektes, die op hogere leeftijd steeds meer voorkomen, ook bij proefdieren. Hieraan werd in de experimenten in dit proefschrift dan ook veel aandacht besteed.
De stand van zaken voor wat betreft het in de vakliteratuur gepubliceerde onderzoek naar de invloed van de leeftijd op de farmacodynamiek van geneesmiddelen met een werking op de hersenen wordt in hoofdstuk 1 beschreven. Uit het hele scala van deze geneesmiddelen wordt voornamelijk aan twee groepen, de zgn. barbituraten en de eerder genoemde benzodiazepines veel aandacht besteed. De groep barbituraten bevat o.a. geneesmiddelen die toegepast kunnen worden als slaapmiddel of als bescherming tegen epilepsie. Het onderzoek, dat wordt besproken in hoofdstuk 1, kan ingedeeld worden in drie categorieën: onderzoek in de mens, onderzoek in proefdieren (beide *in vivo* onderzoek genoemd, omdat in het gehele levende organisme onderzoek wordt gedaan) en onderzoek in hersengedeeltes, die geïsoleerd zijn uit de hersenen van proefdieren (*in vitro* onderzoek genoemd). De onderzoeken in de mens en in proefdieren laten voor verschillende barbituraten en benzodiazepines een verlaagde dosisbehoefte zien op oudere leeftijd. Er zijn aanwijzingen dat dit o.a. zou kunnen komen door een verandering in farmacodynamiek (hoewel dit niet uit alle onderzoeken blijkt). De hersenen zouden gevoeliger zijn geworden voor

de werking van deze geneesmiddelen. Dat wil zeggen dat bij een kleinere hoeveelheid van het geneesmiddel al het gewenste effekt tot stand wordt gebracht; de werking van de geneesmiddelen is sterker (zoals bijv. het halve slaaptabletje dat voor de oudere mens al genoeg is om de hele nacht op door te slapen). De interpretatie van de resultaten van deze onderzoeken in de vakliteratuur, op grond waarvan door de betreffende onderzoekers wordt geconcludeerd dat de hersenen gevoeliger zijn geworden, is echter niet eenduidig vanwege de manier waarop het onderzoek was opgezet. Vaak is er geen rekening gehouden met farmacokinetische processen, die, zoals al eerder genoemd, van belang zijn voor de interpretatie van gegevens over de farmacodynamiek. Een ander probleem heeft te maken met de manier waarop de werking van de medicijnen is getest. Dat geldt bijv. voor het onderzoeken van het effekt van een geneesmiddel op het geheugen van een mens of een proefdier (tijdelijke geheugenverslechtering kan een bijwerking zijn van geneesmiddelen). Bij die onderzoeken blijkt vaak dat na het slikken van het geneesmiddel het geheugen meer is achteruitgegaan bij een oudere dan bij een jongere. Er wordt dan geconcludeerd dat het medicijn een sterkere werking heeft (een grotere mate van verslechtering van het geheugen) bij de oudere. Vergeten wordt dan wel eens dat ook al vóór toediening van het geneesmiddel het geheugen bij de ouderen slechter was dan bij de jongeren (men zegt dan dat de basislijnwaarde verschilt). Dat werd met behulp van de methodes die in die onderzoeken werden gebruikt om het functioneren van het geheugen te meten geconstateerd. Het verschil in geheugen wordt dan niet veroorzaakt door een sterkere werking van het geneesmiddel bij ouderen, maar door de leeftijd op zich. Bij de onderzoeken met proefdieren (meestal ratten of muizen) bestaat nog een probleem. De gezondheidstoestand van de dieren wordt meestal niet vastgesteld. Het gevolg is, dat de dieren (vooral de oudere dieren) ziektes kunnen hebben die ook een waargenomen sterkere werking van medicijnen kunnen veroorzaken. In de derde categorie onderzoek, die waarin hersengedeeltes worden onderzocht, wordt geprobeerd om het mechanisme van een eventuele leeftijdsgerelateerde verandering in farmacodynamiek te achterhalen. Er wordt dan gekeken naar de binding van het geneesmiddel aan de receptor en de daardoor in gang gezette biologische/chemische processen, die uiteindelijk tot het effekt van het geneesmiddel leiden. De resultaten van deze onderzoeken zijn echter tegenstrijdig en geven daardoor geen uitsluitsel over het mechanisme van farmacodynamische veranderingen tijdens veroudering.

Bovendien wordt op deze manier maar een deel van alle relevante processen onderzocht. De conclusie van hoofdstuk 1 is dan ook dat er meer onderzoek nodig is naar de invloed van veroudering op de farmacodynamiek van geneesmiddelen met een werking op de hersenen. De onderzoeken zouden uitgevoerd moeten worden *in vivo* (eerst in proefdieren en later wellicht ook in de mens) en er zou rekening gehouden moeten worden met de gezondheidstoestand van het dier of de mens en met leeftijdsgerelateerde veranderingen in farmacokinetiek en in basislijnwaarden. Belangrijke aspecten van de methodes, die nodig zijn om dit soort onderzoek uit te kunnen voeren zijn beschreven in hoofdstuk 2.

I.3. Toegepaste technieken

I.3.1. Verouderingsaspecten

Bij verouderingsonderzoek in de rat als model voor de mens is het belangrijk om over gegevens ten aanzien van overlevingscurves te beschikken. Zulke curves geven aan hoeveel procent van de ratten op een bepaalde leeftijd nog in leven is. Door in deze curves bijv. de 50% overleving (de gemiddelde levensduur) af te lezen en die te vergelijken met de gemiddelde levensduur van de mens, kan een idee verkregen worden over de verhouding tussen de leeftijd van een rat en die van de mens. Daar de belangrijkste veranderingen tijdens veroudering vaak pas optreden op hoge leeftijd is het belangrijk om ook hoogbejaarde ratten te onderzoeken. De leeftijd waarop een verandering begint kan anders zijn voor verschillende te onderzoeken processen. Bovendien kan de werking van een bepaald proces bijv. gedurende de eerste helft van het leven steeds beter worden en gedurende de tweede helft steeds slechter. Daarom is het belangrijk om niet alleen een groep jonge en een groep oude ratten te onderzoeken, maar ook een aantal leeftijdsgroepen daartussen. In elk onderzoek beschreven in dit proefschrift zijn variërend tussen de vier en zeven groepen mannelijke BN/BiRij ratten gebruikt met leeftijden tussen 3 en 37 maanden (vergelijkbaar met ongeveer 7 en 87 mensenjaren). BN/BiRij ratten zijn Brown Norway ('bruin Noorwegen') ratten, die in Rijswijk speciaal worden gefokt voor onderzoek. De letters Bi staan voor de plaats waar de fokkers uit Rijswijk de ratten vandaan hebben.
Zoals al eerder gezegd, moet bij verouderingsonderzoek rekening worden gehouden met de gezondsheidstoestand van de proefdieren. Daarom werden in alle experimenten van alle ratten een aantal klinisch-biochemische gegevens verzameld (met behulp van metingen in bloed van bijv. suiker en

bloedeiwitten en in urine van bijv. zouten) en werden na het experiment een aantal organen en weefsels onderzocht door een onafhankelijke patholoog (ziektenkundige), die op grond van al deze informatie beoordeelde of een rat aan een ernstige ziekte leed of niet. Zieke ratten werden weggelaten bij het interpreteren van de resultaten van de farmacokinetische en farmacodynamische onderzoeken. Als er echter in een onderzoek veel dieren ziek bleken te zijn kon worden bekeken of de aanwezigheid van ziekte invloed had op de resultaten van het farmacokinetisch en het farmacodynamisch onderzoek. Dit kun je niet doen op grond van enkele zieke dieren omdat dan een gevonden verschil toeval kan zijn net voor die enkele ratten.

Verouderingsonderzoek kan in principe op twee verschillende manieren worden uitgevoerd: volgens een longitudinale onderzoeksopzet of volgens een cross-sectionele opzet. Bij de eerstgenoemde opzet wordt elk proefdier uit een grote groep verschillende malen tijdens zijn leven onderzocht. Het onderzoek duurt dan net zo lang als de 'sterkste' ratten leven (ongeveer drie jaar). Wanneer volgens een cross-sectionele opzet wordt gewerkt, wordt een aantal groepen ratten van verschillende leeftijden binnen korte tijd (bijv. binnen één maand) onderzocht. Elke rat wordt dan slechts één keer onderzocht. Het belangrijkste voordeel van de longitudinale onderzoeksopzet is het feit dat de kans om leeftijdsgerelateerde veranderingen te ontdekken groter is dan bij de cross-sectionele opzet. Een onderzoek volgens de cross-sectionele opzet kan echter in veel kortere tijd worden uitgevoerd dan een onderzoek volgens de longitudinale opzet. Daardoor kunnen de omstandigheden waaronder het onderzoek wordt uitgevoerd (bijv. de leefomstandigheden van de ratten, de onderzoekers die bij het onderzoek betrokken zijn, de gebruikte apparatuur) beter constant worden gehouden. Dat is weer het belangrijkste voordeel van de cross-sectionele opzet. Alle onderzoeken in dit proefschrift, behalve één, zijn uitgevoerd volgens een cross-sectionele opzet. In dat ene onderzoek werd een nieuwe opzet, de zgn. 'pseudo'-longitudinale opzet gekozen. Met deze nieuwe opzet worden zowel enkele belangrijke nadelen van de cross-sectionele opzet als nadelen van de gewone longitudinale opzet omzeild. In de 'pseudo'-longitudinale opzet werd een groep ratten, die allemaal in een tijdsperiode van dezelfde twee weken geboren waren, gereserveerd voor het onderzoek en op verschillende leeftijden werd één subgroepje onderzocht. Elk subgroepje werd dus maar één keer onderzocht. Dit onderzoek zal verderop uitgebreid worden

besproken.

I.3.2. Farmacodynamiek

Om de farmacodynamiek van geneesmiddelen te kunnen bestuderen is een methode nodig om de mate van werking van het geneesmiddel te meten. Voor onze onderzoeken zijn in totaal drie verschillende technieken toegepast. In de eerste methode wordt het narcotisch effekt gemeten m.b.v de zgn. oprichtreflex. Via een slangetje, dat operatief is aangebracht in een bloedvat van de rat, wordt langzaam (als een infuus) een geneesmiddel toegediend dat de rat onder narcose brengt. In het begin loopt de rat nog rond in zijn kooi tot hij langzaam slaperig wordt. Dan wordt hij op zijn rug op een verwarmd matje gelegd (infuus blijft door gaan). Regelmatig wordt een prikkel aan de staart toegediend. Als de rat nog niet goed onder narcose is, trekt hij dan reflexmatig zijn staart terug. Zodra dit niet meer gebeurt is het zgn. punt van verlies van oprichtreflex bereikt en dan wordt het infuus gestopt. Vervolgens wordt er bekeken hoeveel geneesmiddel (narcosemiddel) er is toegediend om dit punt te bereiken. Dit wordt in ratten van verschillende leeftijden gedaan. Door op het punt van verlies van oprichtreflex ook nog de hoeveelheid geneesmiddel die dan aanwezig is in de hersen/ruggemergvloeistof te meten kan iets worden gezegd over de gevoeligheid van de hersenen voor dat geneesmiddel. Dezelfde techniek kan tevens worden gebruikt door een hoge dosis van het geneesmiddel toe te dienen, waardoor de rat meteen onder narcose is. Dan wordt tijdens de narcose het punt bepaald waarop de oprichtreflex weer terugkomt. Deze techniek werd toegepast in hoofdstuk 6, de eerstgenoemde in de hoofdstukken 4 en 5.

De tweede techniek die is gebruikt om de mate van werking te meten berust op het meten van electrische signalen op de hersenschors ('hersengolven'; in medische termen het dit het electroencephalogram, afgekort als EEG). Wanneer een geneesmiddel met een werking op de hersenen aan een mens of een proefdier wordt toegediend, treden er veranderingen op in het patroon van de electrische signalen. Voor de geneesmiddelen, waarvan de onderzoeken zijn beschreven in hoofdstuk 7 en 8, is de omvang van de veranderingen in het EEG (mogelijk) een maat voor de werking van het geneesmiddel. Na toediening van een geneesmiddel kan het EEG continu worden gemeten. Bovendien kan tijdens deze meting op verschillende tijdstippen een beetje bloed afgenomen worden via een slangetje in een bloedvat van de rat. Door achteraf te meten hoeveel geneesmiddel er in die

bloedmonsters aanwezig is, kan een grafiek worden gemaakt waarin voor de verschillende tijdstippen de hoeveelheid geneesmiddel in het bloed en de bijbehorende verandering in het EEG (de mate van werking van het geneesmiddel) worden uitgezet. Met behulp van deze grafiek kan dan iets worden gezegd over de gevoeligheid van de hersenen voor het betreffende geneesmiddel. Deze onderzoeken waarbij de werking van een geneesmiddel wordt gemeten met behulp van het EEG kunnen op een vergelijkbare wijze worden toegepast bij mensen. Daarom is deze methode ook geschikt om te bekijken of de resultaten in de rat vergelijkbaar zijn met die in de mens. Omdat de rat gebruikt is als model voor de mens, is dat natuurlijk wel belangrijk.

De derde techniek is een zgn. directe corticale stimulatie techniek, die wordt toegepast om de beschermende werking van geneesmiddelen tegen epileptische aanvallen te meten. Omdat onze BN/BiRij ratten niet spontaan epileptische aanvallen krijgen, moet er een methode worden gebruikt om het begin van een epileptische aanval na te bootsen. Een electrisch stroompje, dat langzaam in sterkte toeneemt, wordt op metaaldraadjes gezet, die operatief in de schedel van de rat zijn vastgemaakt. Eerst merkt de rat daar nog niets van. Als de stroomsterkte echter een bepaalde drempelwaarde bereikt heeft, vertoont de rat het eerste verschijnsel van een epileptische aanval (hij trekt met een voorpoot). Dan wordt de stroom meteen stopgezet, waardoor ook de aanval niet verder gaat. De rat gaat daarna weer verder waarmee hij bezig was (snuffelen, lopen, slapen). Hieruit blijkt dat hij geen negatieve gevolgen oploopt van deze procedure. De procedure kan dan ook regelmatig worden herhaald in dezelfde rat. Wanneer nu een geneesmiddel met een beschermende werking tegen epileptische aanvallen (een zgn. anticonvulsivum) aan de rat wordt toegediend, moet een hoger stroompje worden toegediend om het trekken met de voorpoot teweeg te brengen. De hoeveelheid stroom die méér toegediend moet worden is een maat voor de beschermende werking van het anticonvulsivum. Na toediening van een anticonvulsivum aan de rat kan regelmatig de drempelstroom worden gemeten en kunnen ook regelmatig bloedmonsters worden genomen. Net zoals eerder beschreven voor het EEG, kan dan een grafiek worden gemaakt met gegevens over de hoeveelheid geneesmiddel in het bloed en de mate van anticonvulsieve werking, die weer iets zegt over de gevoeligheid van de hersenen voor het geneesmiddel. Door dit in ratten van verschillende leeftijden te doen kan de invloed van veroudering op de gevoeligheid van de

hersenen voor die geneesmiddelen worden onderzocht.

In hoofdstuk 3 worden de onderzoeken, beschreven in dit proefschrift, kort ingeleid. De invloed van veroudering op de farmacodynamiek werd bestudeerd voor twee narcosemiddelen (fenobarbital en heptabarbital) met behulp van de techniek van de oprichtreflex. Verder werd voor twee geneesmiddelen (heptabarbital en midazolam) de hersengevoeligheid voor EEG-effekten onderzocht in ratten van verschillende leeftijden. Tenslotte is de invloed van veroudering gemeten op de farmacodynamiek van twee geneesmiddelen tegen epilepsie (oxazepam en natriumvalproaat).

Deel II. Narcotische effekten

In hoofdstuk 4 wordt een onderzoek beschreven waarin de het narcotisch effekt van fenobarbital werd bestudeerd in vijf groepen ratten van verschillende leeftijden. Er werd een afname gevonden voor de dosisbehoefte van 30% tussen de leeftijden 4 en 36 maanden, die werd veroorzaakt door een ongeveer even grote toename in de hersengevoeligheid voor het narcotisch effekt. In de farmacokinetiek werden geen belangrijke leeftijdsgerelateerde veranderingen gemeten. Uit vakliteratuur was bekend dat nierziekte ook kan leiden tot hogere hersengevoeligheid voor het narcotisch effekt van fenobarbital. Aangezien de werking van de nieren achteruit kan gaan tijdens veroudering is onderzocht of de toegenomen hersengevoeligheid misschien samenhing met een verslechtering van de nierfunktie in plaats van door de leeftijd zelf. Dit bleek niet het geval te zijn; de toegenomen hersengevoeligheid was dus hoogst waarschijnlijk een gevolg van veroudering.
Aangezien voor de studie in hoofdstuk 4 een cross-sectionele onderzoeksopzet was gebruikt, is het onderzoek uitgevoerd in verschillende groepen ratten van verschillende leeftijden. Het verschil tussen deze groepen is niet alleen de leeftijd, maar ook het feit dat ze in verschillende maanden en jaren geboren zijn. Deze zgn. cohortverschillen (een cohort is een groep ratten die allemaal in dezelfde korte periode geboren zijn en onder gelijke omstandigheden zijn opgegroeid) zouden in theorie ook de verklaring kunnen zijn van de in hoofdstuk 4 gevonden verschillen tussen de groepen ratten van verschillende leeftijden. Daarom is een vergelijkbaar onderzoek gedaan met behulp van een 'pseudo'-longitudinale opzet (hoofdstuk 5). Zoals reeds eerder

genoemd is, werden in deze opzet in twee opeenvolgende weken geboren ratten gereserveerd voor het onderzoek en op verschillende leeftijden werd een subgroepje onderzocht. In deze opzet kan er geen sprake zijn van cohortverschillen. De gewone longitudinale opzet, waarin elke rat meerdere malen tijdens zijn leven wordt onderzocht, heeft een aantal belangrijke nadelen heeft. Daarom is er voor het onderzoek in hoofdstuk 5 gekozen voor de 'pseudo'-longitudinale opzet. Het is niet met zekerheid uit te sluiten dat de uitvoering van het onderzoek zelf blijvende gevolgen heeft voor een rat. Deze gevolgen kunnen de resultaten van een volgend experiment beïnvloeden. Bovendien kan niet na elk onderzoek in een dier de gezondheidstoestand grondig worden uitgezocht, omdat, zoals uit het onderzoek beschreven in hoofdstuk 6 bleek, daarvoor organen en weefsels uit het dier moeten worden gehaald en dat kan uiteraard slechts éénmaal in dezelfde rat. Uit het 'pseudo'-longitudinale onderzoek in hoofdstuk 5 bleek, net zoals uit het cross-sectionele onderzoek in hoofdstuk 4, dat de hersengevoeligheid voor het narcotisch effekt toeneemt met de leeftijd. Hiermee is aangetoond dat het resultaat van het onderzoek in hoofdstuk 4 niet het gevolg is van cohortverschillen, maar echt van veroudering. Bovendien lijkt de 'pseudo'-longitudinale opzet, die niet eerder is gebruikt, een interessante nieuwe onderzoeksopzet te zijn.

Bij een onderzoek naar de invloed van de leeftijd op de farmacodynamiek van het narcotisch effekt van heptabarbital bleken relatief veel van de gebruikte ratten één of meerdere ernstige ziektes onder de leden te hebben (hoofdstuk 6). Daarom werden de resultaten van dat onderzoek niet alleen geanalyseerd met uitsluiting, maar ook met insluiting van de zieke ratten.
voor de 31 maanden oude ratten werden bovendien de resultaten, verkregen met zieke ratten, vergeleken met die verkregen met alleen gezonde ratten. Bij insluiting van alle dieren bleek na toediening van een vaste dosis van heptabarbital de duur van de narcose langer te zijn in oudere ratten. De oorzaak hiervan bleek te zijn dat in oudere ratten het narcosemiddel langzamer door de lever wordt afgebroken (een leeftijdsgerelateerde verandering in de farmacokinetiek dus). Farmacodynamische verschillen werden niet gevonden. Wanneer de zieke dieren werden uitgesloten, werden echter niet meer zulke grote farmacokinetische verschillen geconstateerd. De hersengevoeligheid daarentegen bleek toe te nemen (met ongeveer 30%) tussen de leeftijden 24 en 31 maanden. Een heel ander resultaat dus. Hieruit

blijkt hoe belangrijk het is om de gezondheidstoestand van de ratten goed vast te stellen en vervolgens de ernstig zieke dieren uit te sluiten. Bij het vergelijken van de farmacokinetiek en de farmacodynamiek in de zieke en de gezonde 31 maanden oude ratten bleken tussen deze twee groepen ook verschillen te bestaan in de mate van afbraak van heptabarbital door de lever en in de hersengevoeligheid voor dit narcosemiddel (beide waren lager in de zieke ratten). Zoals eerder vermeld werd door een onafhankelijke patholoog beslist of een rat ernstig ziek was op grond van klinisch-biochemische gegevens en onderzoek van een aantal organen en weefsels. Dit laatste kan alleen maar na de dood van een rat en het zou natuurlijk geschikter zijn als alleen de klinisch-biochemische gegevens voldoende zouden zeggen over de ziekte van een rat. Helaas bleek dat niet het geval te zijn.

Deel III. Effekten op de 'hersengolven' (EEG)

In een onderzoek naar de invloed van veroudering op de farmacodynamiek van de EEG-effekten van het narcosemiddel heptabarbital bleek dat de hersengevoeligheid voor deze effekten niet verandert met toenemende leeftijd (hoofdstuk 7). De resultaten van dit onderzoek, waarin het effekt van heptabarbital op het EEG werd gemeten kunnen nu worden vergeleken met die waarin het narcotisch effekt van heptabarbital werd gemeten (hoofdstuk 6). De resultaten komen echter niet overeen. Bij het meten van de EEG-effekten werd geen invloed van veroudering op de farmacodynamiek gevonden, terwijl in het onderzoek naar het narcotisch effekt van heptabarbital een toegenomen hersengevoeligheid werd gevonden in oudere ratten. Een verklaring hiervoor zou als volgt kunnen zijn. Het is (nog) niet helemaal duidelijk met welke klinische werking (een werking waarvoor het geneesmiddel wordt toegepast bij patiënten) het EEG-effekt van heptabarbital overeenkomt, maar waarschijnlijk heeft het wel iets met narcose te maken. Zoals al eerder is genoemd kunnen regelmechanismen in het lichaam betrokken zijn bij het tot stand komen van de werking van een geneesmiddel. Het is voorstelbaar dat bij het tot stand komen van het narcotisch effekt complexere (tegen)regelmechanismen betrokken zijn dan bij het EEG-effekt en wel om de volgende reden: bij het meten van narcose wordt naar het héle dier wordt gekeken en het EEG wordt alleen in de hersenen gemeten (voor de volledigheid moet hier aan worden toegevoegd dat mechanismen in de

rest van het lichaam ook van invloed kunnen zijn op de 'hersengolven'). Nu is uit de vakliteratuur is bekend dat regelmechanismen niet meer optimaal functioneren bij ouderen. Daarom kan de betrokkenheid van complexere regelmechanismen bij het tot stand komen van het narcotisch effekt een verklaring zijn voor het feit dat er met het ouder worden wel iets verandert in de gevoeligheid voor het narcotisch effekt van heptabarbital (omdat die regelmechanismen slechter functioneren) en niet in die voor het EEG-effekt (omdat de minder complexe regelmechanismen goed blijven functioneren tijdens veroudering).

Naast heptabarbital werd ook van midazolam de hersengevoeligheid voor het EEG-effekt gemeten in ratten van verschillende leeftijden (hoofdstuk 8). Midazolam wordt veelvuldig gebruikt voor narcose in ziekenhuizen. In Engeland werd enkele jaren geleden in 800 patiënten in leeftijd variërend tussen 15 en 90 jaar de dosisbehoefte gemeten nodig voor narcose voor een 'kijkoperatie' (in het maagdarmkanaal). De dosisbehoefte bleek met maar liefst 80% af te nemen tussen deze leeftijden; de grootste afname werd geconstateerd bij 65-plussers. In onze rattenstudie werden geen farmacokinetische veranderingen geconstateerd en de hersengevoeligheid leek met ongeveer 50% toe te nemen tussen de leeftijden 29 en 36 maanden (vergelijkbaar met ongeveer 68 en 84 mensenjaren). Binnen een aantal groepen ratten van dezelfde leeftijd werden ook grote onderlinge verschillen in hersengevoeligheid gevonden. Dit geeft aan dat er naast de leeftijd voor midazolam nog andere factoren moeten zijn die leiden tot verschillen in dosisbehoefte tussen patiënten.

Deel IV. Anticonvulsieve effekten

Bij het herhaaldelijk meten van het anticonvulsieve effekt (bescherming tegen epileptische aanvallen) na toediening van het geneesmiddel oxazepam aan jonge BN/BiRij ratten werd een opvallend verschijnsel geconstateerd (hoofdstuk 9). Vlak na toediening van oxazepam is de hoeveelheid van dit geneesmiddel in het bloed het hoogst en daarom is dan ook de werking het sterkst. Voornamelijk als gevolg van afbraak door de lever daalt de hoeveelheid van deze stof in het bloed en zo ook het effekt (de beschermende werking tegen een epileptische aanval wordt steeds minder). Men zou verwachten dat het anticonvulsieve effekt na verloop van tijd

ophoudt als ook bijna al het geneesmiddel uit het lichaam verdwenen is. Bij de BN/BiRij ratten blijkt echter dat na toediening van een bepaalde dosis oxazepam de bescherming tegen een epileptische aanval steeds minder wordt (sneller dan verwacht op grond van resultaten in een andere stam ratten) en na verloop van tijd lijkt het alsof oxazepam niet meer beschermt tegen een epileptische aanval, maar zelf een aanval opwekt. De snellere afname van het effekt dan verwacht suggereert dat de ratten heel snel gewend raken aan het geneesmiddel, waardoor het niet meer zo goed werkt. Het 'zelf opwekken' van een epileptische aanval lijkt op een ontwenningsverschijnsel (afkickverschijnsel) tijdens de afname (als gevolg van afbraak door de lever) van de hoeveelheid oxazepam die nog in het lichaam aanwezig is. De verschijnselen van gewenning en ontwenning hebben te maken met (tegen)regelmechanismen in het lichaam van de rat. Bij de oudste ratten worden deze verschijnselen niet meer gevonden. Hier lijkt het er dus weer op dat regelmechanismen het af laten weten in de oudere dieren. In de farmacokinetische processen werden overigens voor oxazepam geen veranderingen gevonden met toenemende leeftijd.

Zoals al eerder genoemd oefenen de (meeste) geneesmiddelen die in het kader van dit proefschrift zijn onderzocht hun werking uit door eerst te binden aan een specifiek eiwit in de hersenen (de receptor). In de hersenen van de ratten van het oxazepamonderzoek werd na dit onderzoek *in vitro* de binding van een vergelijkbaar geneesmiddel aan de receptor bepaald. Deze metingen leveren gegevens op over de hoeveelheid bindingsplaatsen op de receptor en over de zgn. affiniteit (neiging tot binding, 'aantrekkingskracht') van een geneesmiddel voor de receptor. Er bleken geen leeftijdsgerelateerde veranderingen op te treden in de binding aan de receptor. Dit bevestigt dat de veranderde gevoeligheid inderdaad kan samenhangen met een verandering in regelmechanismen.

In het laatste onderzoek (hoofdstuk 10) werd het anticonvulsieve effekt van natriumvalproaat gemeten in groepen ratten van zeven verschillende leeftijden. Natriumvalproaat is een geneesmiddel dat veel wordt gebruikt voor de behandeling van epilepsie. Het geneesmiddel werkt via beïnvloeding van bepaalde processen in de hersenen. Twee leeftijdsgroepen meer dan in de meeste andere onderzoeken. Deze extra leeftijdsgroepen bestonden uit jong volwassen ratten en ratten van middelbare leeftijd. De reden hiervoor ligt in de resultaten van andere onderzoeken beschreven in de vakliteratuur. Met het ouder worden werd bij de mens een lagere gevoeligheid gevonden voor

de werking van natriumvalproaat op een systeem in de hersenen (hypothalamus/hypofyse systeem). Deze werking verloopt via biologisch/chemische reakties in de hersenen, die ook een rol spelen bij de anticonvulsieve werking van natriumvalproaat. Ook is uit de vakliteratuur bekend dat, in tegenstelling tot veel andere geneesmiddelen, juist bij jongeren die dit geneesmiddel gebruikten veel bijwerkingen werden geconstateerd in vergelijking met ouderen. Daarom wilden we bij ons onderzoek extra aandacht geven aan de wat lagere leeftijden. De resultaten van ons onderzoek naar de invloed van veroudering op de farmacodynamiek van het anticonvulsieve effekt waren anders dan we op grond van de genoemde gegevens uit de vakliteratuur hadden verwacht. De oudere ratten bleken een lagere hoeveelheid natriumvalproaat in het bloed nodig te hebben voor bescherming tegen een epileptische aanval. In de hersenen van oudere ratten wordt dus met een lagere hoeveelheid dan bij jongere ratten nodig is al hetzelfde effekt bereikt. Dit suggereert een toenemende gevoeligheid van de hersenen voor de anticonvulsieve werking van natriumvalproaat tijdens het ouder worden. Dit resultaat is dus vergelijkbaar met dat voor het anticonvulsieve effekt van oxazepam, waar ook bleek dat oudere ratten een lagere hoeveelheid van dat geneesmiddel nodig hebben voor bescherming tegen epileptische aanvallen.

Deel V. Conclusies en perspectieven voor toekomstig onderzoek

In hoofdstuk 11 worden alle onderzoeken in het algemeen bediscussiëerd en vergeleken.

V.1. De invloed van ziekte

Door alle gegevens van alle zieke ratten te verzamelen, wilden we trachten te achterhalen welke ernstige ziektes wél een invloed hebben op de farmacokinetiek en de farmacodynamiek en welke níet. Er kan echter geen algemene conclusie worden getrokken, omdat de 26 zieke ratten allerlei verschillende ziektes hadden en ook de ernst van de ziektes nogal verschilde. Er zijn dus niet veel ratten met dezelfde ziekte in dezelfde mate. Om echter een goede conclusie te kunnen trekken zijn meer ratten met dezelfde ziekte nodig. Bovendien lijdt één rat regelmatig aan meerdere ziektes en dan weet je niet aan welke van die ziektes een eventuele afwijking in farmacokinetiek

of farmacodynamiek is toe te schrijven. Het bleek dat het soort ziektes dat werd gevonden niet gelijk is voor alle cohorten ratten. Om alle zieke ratten op te sporen (en uit te kunnen sluiten bij de interpretatie van de resultaten van een onderzoek) is het dus belangrijk om voor elk onderzoek toch weer bij het vaststellen van de gezondheidstoestand van de ratten rekening te houden met alle ziektes, zelfs als een bepaalde ziekte in een aantal cohorten niet meer is geconstateerd. In hoofdstuk 6 werd geconcludeerd dat alleen klinisch-biochemische gegevens niet voldoende zijn om te kunnen concluderen of een rat ernstig ziek is of niet, maar dat daarnaast onderzoek van een aantal relevante organen en weefsels noodzakelijk is. Deze conclusie wordt bevestigd door de resultaten van andere onderzoeken in dit proefschrift.

V.2. De te onderzoeken leeftijden

In hoofdstuk 2 werd als algemene 'regel' voor verouderingsonderzoek genoemd, dat ook echte oude ratten (van de leeftijden rond die welke 50% en rond die welke 10% van de ratten haalt, dat is voor onze ratten rond 32 en 38 maanden) onderzocht moeten worden. Deze 'regel' is als zodanig, omdat de belangrijkste veranderingen vaak pas op hoge leeftijd optreden. In onze onderzoeken werd dit inderdaad bevestigd. Bovendien bleek dat het belangrijk was om meer dan twee leeftijden ('jong' en 'oud') te onderzoeken, omdat de leeftijd waarop een bepaalde verandering begint nogal eens verschillend was. Door alleen 'jong' en 'oud' te onderzoeken kom je daar natuurlijk nooit achter.

V.3. De invloed van veroudering op de mate van werking van narcosemiddelen en geneesmiddelen tegen epilepsie

Er kan geen algemene conclusie worden getrokken over de invloed van veroudering op de farmacodynamiek van narcosemiddelen en anticonvulsiva. We hebben een leeftijdsgerelateerde toename in hersengevoeligheid gevonden voor de narcotische werking van fenobarbital en heptabarbital en voor de anticonvulsieve werking van oxazepam en natriumvalproaat. Voor oxazepam lijkt deze toename het gevolg te zijn van een verminderde werking van regelmechanismen in het lichaam die betrokken zijn bij het tot stand komen van de anticonvulsieve werking. Voor natriumvalproaat laat de manier waarop het onderzoek is opgezet geen conclusies toe over deze regelmechanismen. De hersengevoeligheid voor de EEG-effekten van

heptabarbital veranderde niet tijdens het ouder worden; die voor de EEG-effekten van midazolam leek toe te nemen op hoge leeftijd. Aangezien er geen algemene conclusies getrokken kunnen worden, zal de invloed van veroudering op de farmacodynamiek voor meer geneesmiddelen apart onderzocht moeten worden volgens methodes zoals die zijn toegepast in ons onderzoek. De resultaten van ons onderzoek, gecombineerd met de gegevens uit de vakliteratuur over de binding van geneesmiddelen aan hun receptor en de chemische reakties die daarna optreden, suggereren dat de achteruitgang van (tegen)regelmechanismen een belangrijke oorzaak is voor leeftijdsgerelateerde toename in de hersengevoeligheid voor de werking van narcosemiddelen (sedativa) en geneesmiddelen tegen epilepsie (anticonvulsiva). Dit laat zien dat het bij het onderzoeken van deze veranderingen belangrijk is om zodanige methodes te kiezen, dat zoveel mogelijk regelmechanismen worden 'meegemeten'. Dat kan alleen maar bij *in vivo* onderzoek.

V.4. Perspectieven voor verder onderzoek

Een uitdaging voor toekomstig onderzoek is het vaststellen van de voorspellende waarde van ons verouderingsonderzoek in de rat voor de situatie in de mens. Daarvoor is het nodig om bij de mens *in vivo* farmacodynamische studies te doen volgens de opzet zoals die gebruikt is voor onze rattenstudies. Het meten van EEG-effekten biedt hiertoe een interessante mogelijkheid, omdat EEG-effekten bij de mens en de rat op een vergelijkbare wijze gemeten kunnen worden.
Daar het niet ongewoon is dat oudere mensen een of meerdere ziektes hebben, is het belangrijk om de farmacodynamiek van geneesmiddelen niet alleen te onderzoeken bij gezonde ouderen, maar ook bij zieke ouderen (de zieke ouderen zijn per slot van rekening ook degenen die geneesmiddelen nodig hebben). Doordat de ouderen vaak meerdere ziektes tegelijk hebben, gebruiken ze ook vaak meerdere geneesmiddelen naast elkaar. Omdat geneesmiddelen elkaars werking in het lichaam kunnen beïnvloeden, verdienen deze zgn. geneesmiddelinterakties bij ouderen ook nader onderzoek. Voor deze twee richtingen van toekomstig onderzoek (onderzoek bij zieke ouderen en de geneesmiddelinterakties bij ouderen) zou een nieuwe methode, de zgn. populatiefarmacodynamiek, uitkomst kunnen bieden. Voor deze methode zijn gegevens nodig van een grote heterogene groep personen (een populatie), aan wie het te onderzoeken geneesmiddel is toegediend.

Heterogeen wil in dit opzicht zeggen dat het mensen zijn van verschillende leeftijden, met en zonder ziektes, die wel of geen andere geneesmiddelen gebruiken enzovoorts. Met behulp van deze methode, waarvoor computers met veel geheugenruimte nodig zijn, kan op grond van relatief weinig gegevens per persoon worden onderzocht of bijv. ziekte een invloed heeft op de farmacodynamiek van het toegediende geneesmiddel.
Tenslotte is ook onderzoek naar de mechanismen achter leeftijdsgerelateerde veranderingen in farmacodynamiek in de mens belangrijk. Voor dit soort onderzoek zouden in de toekomst twee nieuwe technieken toegepast kunnen worden, waarmee bepaalde gedeeltes van de hersenen letterlijk in beeld gebracht kunnen worden. Deze technieken, positron emissie tomografie (PET) en enkel foton emissie tomografie (SPET), zijn nu nog in ontwikkeling.

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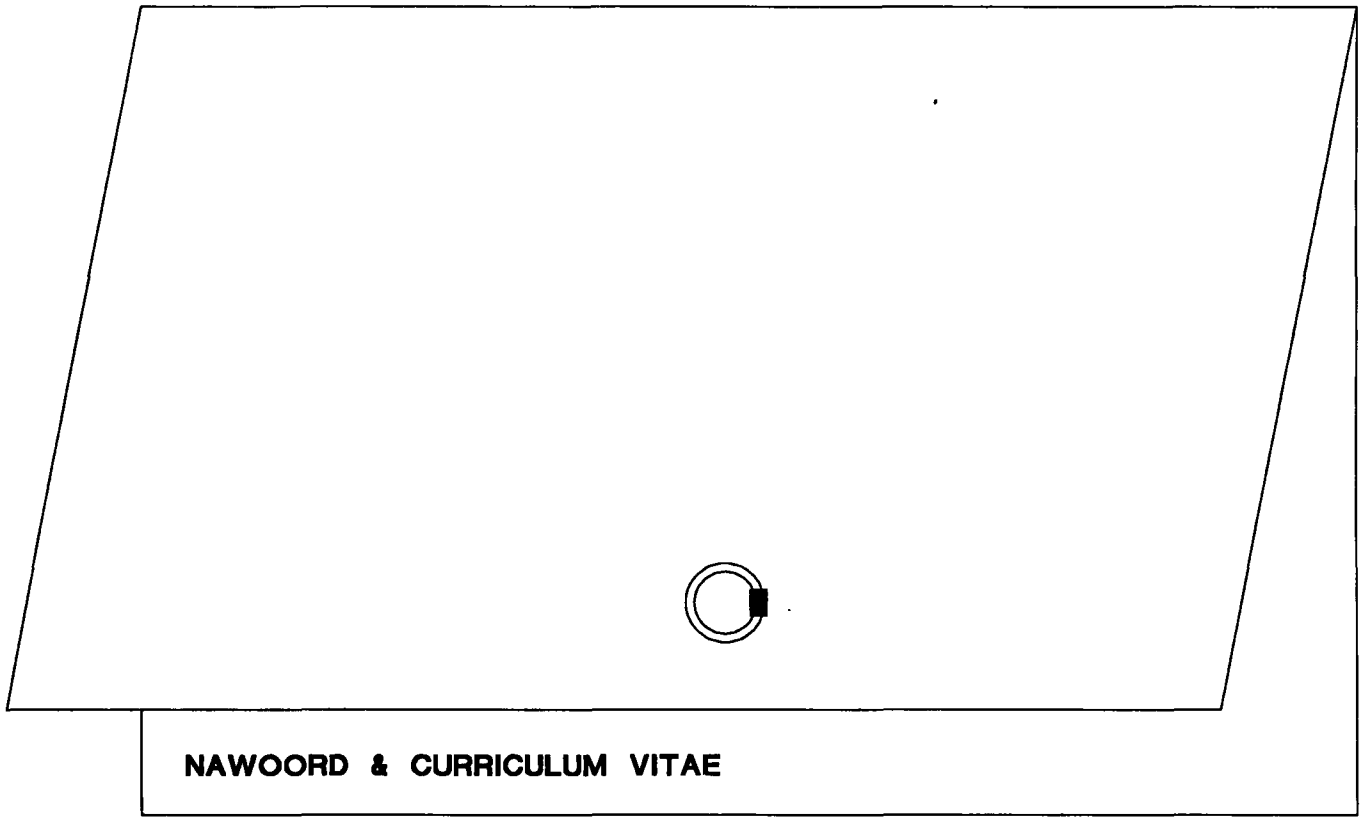
NAWOORD & CURRICULUM VITAE

NAWOORD

Gerontologisch farmacologisch onderzoek vereist de inzet en samenwerking van vele personen uit verschillende disciplines. Daarop is het onderzoek in dit proefschrift zeker geen uitzondering. Daarom ben ik allen zeer erkentelijk die, op welke wijze dan ook, hun bijdrage hebben geleverd aan de totstandkoming van mijn proefschrift. Het is niet mogelijk iedereen bij name te noemen, maar toch wil ik een aantal mensen hier vermelden.
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Annemiek

CURRICULUM VITAE

Annemiek Stijnen werd geboren op 12 april 1964. Na het behalen van het Atheneum-B diploma aan het Bisschoppelijk College in Echt in 1982 werd de studie Farmacie aangevangen aan de Rijksuniversiteit te Leiden. Het propaedeutisch examen werd in augustus 1983 afgelegd, gevolgd door het doctoraalexamen in september 1986. Tijdens de doctoraalstudie werd in het kader van het bijvak Farmacologie een onderzoek verricht naar de bevordering van de rectale absorptie van een antibioticum m.b.v een commercieel verkrijgbaar mengsel van glyceriden, glycerol en octaanzuur onder leiding van dr. A.G. de Boer en drs. E.J. van Hoogdalem.
Van oktober 1986 tot april 1990 was zij als wetenschappelijk onderzoeksmedewerker in dienst van de Nederlandse organisatie voor wetenschappelijk onderzoek (NWO), waarbij het onderzoek werd uitgevoerd bij de sektie Farmacologie van het Centrum voor Bio-Farmaceutische Wetenschappen van de Leidse Universiteit (hoofd: prof. dr. D.D. Breimer) en bij het toenmalige TNO Instituut voor Experimentele Gerontologie in Rijswijk (hoofd: prof. dr. D.L. Knook), hetgeen nu is ondergebracht in het TNO Instituut voor Veroudering en Vaatonderzoek in Leiden. De dagelijkse begeleiding van het promotieonderzoek en het schrijven van dit proefschrift was in handen van dr. M. Danhof en dr. C.F.A. van Bezooijen. In 1988 werd de bevoegdheid voor het verrichten van dierexperimenteel onderzoek conform artikel 9 van de Wet op Dierproeven behaald. De auteur genoot in oktober 1991 het voorrecht haar promotieonderzoek gewaardeerd te mogen zien met de Juniorprijs voor Verouderingsonderzoek.