1975 - EPILEPSYRESEARCH IN THE NETHERLANDS - 1985

QUO VADIS ?

Proceedings of a Symposium organized by
the TNO National Epilepsy Research Committee on March 29, 1985 at Utrecht

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CONTENTS

INTRODUCTION

The symposium from which the papers are presented in this book has originated differently than other symposia on epilepsy. It was organised to reflect on achieved results, not to present breath-taking new discoveries, to mark a point in time, not to start a new era, and to obtain possible future goals for funding in epilepsy research.
Another difference is that it was organised by CLEO-TNO, an advisory committee for funding of epilepsy research, not by the research field itself or affiliated organisations. CLEO-TNO (the National Epilepsy Research Committee of the Netherlands Organization for Applied Scientific Research) is in charge of reviewing research propositions for funding by money which is managed by TNO and is contributed by private as well as public sources.

The theme of the symposium was to present an overview of the achievements obtained by investigations over the last ten years in epilepsy research by experienced experts, together with results from recent CLEO supported investigations.
Because only a limited number of fields can be covered in one day the organizers had decided to center on three topics, i.e. neurophysiological, neurochemical and clinical aspects of epilepsy.
The lectures held on these topics form the main part of these proceedings. They are supplemented by the summaries of 23 poster presentations which were on display during the symposium. Lectures and posters give a good overview of past and present research in epilepsy in The Netherlands.

The participants in the symposium received eight theses which formed the starting point for a forum discussion which had the aim to obtain some insight in the direction of epilepsy research in the future. These theses are presented at the end of this introduction together with a summary of the discussion.

The presentation by Meinardi reviews the subjects and some of the results obtained in the more than 50 CLEO-supported research projects over the last 20 years. It aptly reveals the vastly differing research areas covered, but also shows that the attention has not been evenly distributed over these areas. This last topic was also one of the subjects proposed in the theses.
The predictions Meinardi gives may be worth while reading now and ten years from now as the development of technology as well as medical technique have always been guided by unexpected turns. Hence some of the seemingly logical prospects may not come true while other at this moment rather speculative prognostications on the contrary may work out.

The neurophysiological contributions cover the cellular events occurring in models of experimental epilepsy.
The overview by Lopes da Silva concerns the model in which the brain is gradually kindled into epileptic activity by repeated application of short bursts of minute currents. It describes the gradual changes from normal to epileptic activity within the neural networks of the hippocampus and the possible effects it can have on a.o. memory.

This presentation is well augmented by the work of Voskuyl and Albus who give information on their current work on the effects of various convulsants on the hippocampus, showing that at least two different forms of epileptic activity can be discerned, with probably significance for the understanding of the effect of anti-epileptic substances.

The neurochemical field is presented by an overview by Hommes and Feenstra who on the basis of their own CLEO-supported work and data from literature, make the assumption that a highly localized disturbance of the balance between excitatory and inhibitory amino acid neurotransmitters set into motion a series of events which lead to the development of epilepsy.

The paper by Schrama et al. focusses on the molecular mechanisms underlying epileptogenesis. They show that in different models of epilepsy alterations in the phosphorylation of several neural proteins run parallel to electrophysiological changes which may be relevant to a better understanding of the efficacy of antiepileptic drugs.

The clinical presentation by Overweg et al. centres on the question of drug withdrawal and the prediction of recurrence of epilepsy. Their study shows that the number of different antiepileptic drugs, their cumulative serum level and the subjects age at the last seizure are the main variables predictive for the effect of drug withdrawal, whereas the EEG is of negligible prognostic value.

Nearly all papers are based on original work, for the majority supported by CLEO. Together they give an overview of what has been achieved in the last ten years of epilepsy research in The Netherlands.

Theses

1. Funding of epilepsy research must be preceded by inventarisation.

2. Reflecting on need to treat is better than treating without knowledge of need.

3. Because a strong research program in forensic and psychosocial fields is not in existence, these topics are rarely considered as research goals.

4. Epilepsy Centres are essential for epilepsy research but as such insufficiently used.

5. The existence of a national computerized case-register of patients with epilepsy is an important databank for epilepsy research.

6. Quantitative EEG analysis methods form a separate part in the diagnostic procedures in clinical epilepsy.

7. For the development of an unambiguous model for epileptogenesis, membrane processes, especially related to CA++-permeability have to be taken into account.

8. Knowledge of endogenous epileptogenetic mechanisms must form the foundation in the quest of new anti-convulsive drugs. It should be the main topic for biochemical research in epilepsy.

Participants in the discussion were:
Drs. E.W. Roscam Abbing M.D., Head department of Health Care, Ministry of Science, Health Care & Culture, President of the forum.
Dr. F. Touw-Otten, Medical Sociologist University of Utrecht and President of CLEO-TNO.
Prof. Dr. F.H. Lopes da Silva M.D., Neuroscientist, Head Department Comparative Anatomy, University of Amsterdam.
Prof. Dr. H. Meinard M.D., Neurologist, Director of the Epilepsy Centre "Meer en Bosch", Heemstede.
Prof. Dr. O.R. Hommes M.D., Neurologist, Head Department Experimental Neurology, University of Nijmegen.
Prof. Dr. A.C. van Huffelen M.D., Clinical neurophysiologist, Head Department Clinical Neurophysiology, University of Utrecht.
Dr. J.L. Blom M.D., Neuroscientist, Head Department Neuroscience Netherlands Institute for Preventive Health Care, Leiden, Coördinator CLEO-TNO.

In a discussion with members of the audience the first thesis led to the explanation that too often research proposals are formulated without sufficient insight in the need for the subject under investigation. This leads to a "crowding" of research proposals in some fields with a relative paucity in others which may impede the progress in epilepsy research as a whole. As example may serve the situation for the neurosurgical treatment of epilepsy for which the interest in The Netherlands is low as compared to other countries. It is brougth forward that in the Dutch situation a deficiency in technical provisions exists for which several factors may be responsible. However, neurosurgical treatment is still performed on a small scale for which a working committee exists which regularly discusses cases suitable for treatment.The activities of the committee are rapidly increasing.

Referring to the third thesis the question was put forward if better and more coherent forensic and psychosocial research into the attitude of the population on a regular base is very much needed as results from this kind of research in the U.S.A. indicate that without specific information to the public the rather negative attitude, expressed in the difficulties encountered by patients with epilepsy when applying for a job, drivers licence, etc., cannot easily be changed. In general this kind of research is useful but it is important also to look into the methods used to remove prejudices. They must be chosen according to the objectives and will therefore differ considerably. It was also pointed out that research into attitude is but one subject in this field. Other, equally important, research areas are the epidemiology of epilepsy, the relation between the needs and the means for therapy, the inventarisation of regulations, if existing, etc. It is important to look for developments in other countries and it is desirable to come to some international cooperation in research. CLEO, however, supports primarily Dutch research. Especially in social and forensic fields it is necessary to develop a research program, after a survey of the needs and the possibilities to conduct such a program, for appropriate funding.

The fourth thesis which put forward that epilepsy centres were insufficiently used for research purposes, formed the basis for a disqussion on funding of research in epilepsy. Scientists and administrators were concerned with the current funding policy which prevented the development of long term planning in epilepsy research due to the fact that on one side grants are awarded for periods of maximally three years while on the other hand epilepsy centres in Holland are financed the same way as general hospitals which does not take into account that research forms an essential part of the tasks of these centres. From the funding side these problems were recognised but also indicated that if granting was

done on a long-time base, no grants would be available for pilot studies and short projects. Also the funding policy in epilepsy shows that applied as well as fundamental research obtains sufficient funds if the quality is excellent. The latter is the most important factor in subsidizing research projects.

The discussion on the fifth thesis showed the difficulties in organizing a useful national case register due to the fact a.o. that the established medical profession and the very young profession of informatics do not easily communicate. Another problem is formed by the protection of privacy of the patients. This problem, which not only concerns patiens with epilepsy, forms a major stumble stone and makes the medical profession reluctant to participate in nationwide databanks on illnesses which contain individual information. It is however important that these problems become solved as the advantage of these information concentrations for research and administration is obvious.

The thesis on quantitative analysis of the EEG as a diagnostic tool led to an interesting discussion in which on one side was argued that these techniques were of no avail for diagnosis, while on the other hand it was said that these techniques are most important at least in certain types of patients. It was concluded that for the practical situation not one or another diagnostic method had to prevail. In the combination of information obtained with different methods the diagnosis must lay.

The discussion on the last two theses was mainly about their apodictical nature. Both expressed a certainty which by several of the discussants was doubted. The importance of the former was actually not the proposed model but the point of view that only models, which take into account all consequences of the hypothesis on which they are built and which are not contrary to experimental data, can give us insight into the basic mechanisms in epileptogenesis which still elude us. Undoubtedly, changes in the regulating proteins for certain membrane receptors in which calcium may play an essential role, are involved in these mechanisms. In fundamental research in epilepsy these kind of models are essential as they can be tested to experimental and clinical data. They will be most important in the development of new drugs. This tied in with the last thesis although there the importance was in the proposed model. It was opposed that this at least had to be nuanced as not all parts of the C.Z.S. show the same tendency to become epileptogenic.
It was also discussed that some of the given arguments can be explained differently. As an example may serve the supposed hypoactivity in glucose metabolism in the area surrounding an epileptic focus. On one side it may be argued that it expresses a

hypometabolism but on the other hand it may be due to a high inhibitory activity. The highly complex cooperation between neurons in a neuronal network makes simple explanations not very likely.

The discussion, centered around the eight theses, showed that many topics are of importance to the field of research in epilepsy, ranging from its financing to its fundamental hypotheses. It is to be hoped that some of the ideas put forward have become reality in ten years time and a next symposium may give some answers to the questions asked on the one today.

Leiden, 1986

Dr. J.L. Blom,
coördinator CLEO-TNO

THE KINDLING MODEL OF EPILEPSY OF THE HIPPOCAMPUS IN THE RAT

F.H. Lopes da Silva

Repeated tetanization at regular intervals i.e. kindling of a brain structure leads ultimately to the development of an epileptogenic focus. This is the so-called kindling model of epilepsy which was for the first time described by Goddard (1967). Several aspects of the phenomenology of the kindling model have been investigated as reviewed by Racine (1978) and McNamara et al. (1980).
In this study, we have tried to reply to two different questions regarding the development of an epileptogenic focus due to kindling:
(i) which are the electrophysiological changes which are responsible for this process?
(ii) can the kindling procedure offer a model not only for motor seizures but also for inter-ictal behavioral deficits?
In this chapter, we will review briefly these two aspects (Part A and B) of our current research, without entering into detailed technical descriptions which are published elsewhere (Wadman et al. 1985; Kamphuis et al. 1985).

Part A
Electrophysiological processes underlying kindling epileptogenesis

In order to study such processes, it is important to choose a suitable brain area where detailed neurophysiological analysis can be carried out. For this purpose, we chose the hippocampus of the rat for a number of reasons:
(i) the hippocampus has a laminated structure which offers the possibility of determining accurately the neuronal sources of field potentials; this is of importance since we wish to find out which synaptic processes are affected by the kindling form of stimulation;
(ii) a good deal of information is available regarding the basic physiology of hippocampal networks of the rat (Leung 1979; Leung et al. 1982; Wadman et al. 1983, 1985) and the cellular properties of hippocampal cells (Schwartzkroin 1975; Andersen et al. 1980; Knowles et al. 1984);
(iii) there is also a wealth of information concerning the histological structure of the hippocampus not only at light microscopical level but also at the ultrastrucural level (Ribak and Anderson 1980, Ribak 1985) and in relation to different systems of neurotransmitters (Storm-Mathisen and Ottersen 1984).

Material and Methods

Wistar male rats were chronically inplanted under pentobarbitone anesthesia wih stainless steel electrodes (diameter 100 μm, cut sharp) in the dorsal hippocampus for recording and stimulation. The electrodes were placed in such a way that the Schaffer collaterals in CA_1 fields were optimally stimulated. Single pulse stimulation of this pathway evoked a field potential which reached maximal negativity (corresponding to the extracellular EPSP) at the level of stratum radiatum and was positive going at the stratum pyramidale. Usually, a bundle consisting of three electrodes was used for stimulating and at least two electrodes were used for recording: one was placed in the stratum radiatum and the other above it in the stratum pyramidale. Reference electrodes were screws placed in the skull. The electrodes were cemented in place and connected to a socket which was fixed to the skull. On the plug FET amplifiers were mounted, functioning as preamplifiers and connected via a cable to a commutator and standard amplifiers. Recordings werd performed about one week after implantation The signals were sampled by a microcomputer which was programmed for obtaining averages.
The kindling procedure consisted in stimulating the Schaffer collaterals with one s. pulse trains (50 Hz, 0.2 ms duration), two or three times a day at intervals of at least 3 hours. The procedure was carried out every day until generalized convulsions occurred. The stimulus intensity for kindling was usually the same that was necessary to obtain a field potential with an amplitude equal to half of the saturation level.
At the end of an experiment, which usually lasted 4 or 6 weeks, the rats were sacrified and the brain was perfused for further histological investigations. The localization of the electrodes was confirmed histologically.

Results

The kindling stimulus leads to the occurrence of after-discharges (ADs) which tend to increase in length in the course of time. Another EEG phenomenon which is characteristic of the development of an epileptogenic focus is the occurrence of interictal epileptiform transients of different types as already published elsewhere (Wadman et al. 1983). In order to probe the state of excitability of the neuronal network within and around the area where the epileptogenic focus becomes established, we used the method of paired-pulse stimulation (cf. Creager et al. 1980). We paid particular attention to the changes in the corresponding field potentials evoked by Schaffer stimulation as they take place during the kindling process. In Fig. 1 a comparison of the field potentials to paired-pulse stimulation obtained during the

control period (session 1), the middle periode (session 9) and the final period (session 17) is shown.

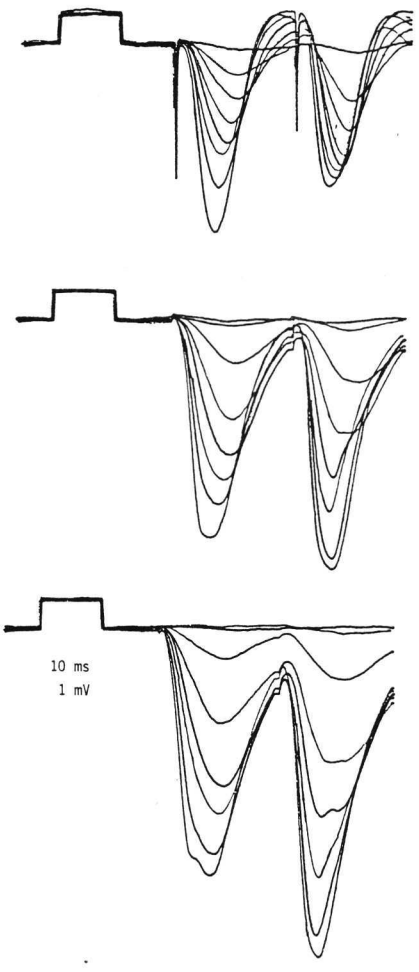

Fig. 1. CA field potentials to paired-pulse stimulation of the Schaffer collaterals. Recording from one electrode placed at the level of the apical dendrites (stratum lacunosum-moleculare) and ipsilaterally to the site of kindling stimulation. Different traces correspond to field potentials evoked by stimuli of different strength. Note the changes in field potentials along kindling sessions (1st, 9th and 17th): (i) the change in the positive-going wave (or late wave in Fig. 2) which occurs after the field EPSP; (ii) the increase in amplitude of the field EPSPs; (iii) the change in the ratio between the amplitude of the field EPSP evoked by the 2nd pulse in relation to that evoked by the 1st.

The main characteristics of paired-pulse responses in the control period were the following:

- the response to the 1st pulse was characterized by an initial negative-going deflection at the level of the stratum radiatum which lasted for 10-12 ms and was followed by a "late" positive-going slower deflection, if the stimulus intensity was above a certain level;
- the response to the 2nd pulse, which was usually given 20 ms after the 1st, showed also a negative-going deflection followed

by a slower component of opposite polarity. At low intensities, the amplitude of the 2nd negative wave was larger than that of the first; this phenomenon is known as paired-pulse facilitation (Creager et al. 1980). At high intensities, however, the 2nd was clearly smaller than the first.

Before examining the changes in these field potentials, we have to understand the basic physiology of these responses. Even at low intensities, one stimulus evokes an EPSP at the level of the synapses formed by the Schaffer collaterals and the apical dendrites of the pyramidal cells (Lopes da Silva et al. 1984). However, this weak stimulus may not be sufficient to provoke cell firing and thus the inhibitory interneurons which are normally excited by collaterals of the axons of CA_1 pyramidal cells may not be activated (Andersen et al. 1964). The "late" positive component occurs at the time where intracellularly the early IPSP can be recorded (Knowles et al. 1984). This inhibitory component will not occur with weak stimuli. The 2nd stimulus may elicit a larger field EPSP probably due to the mobilization of neurotransmitter as currently assumed (Creager et al. 1980). When the strength of the 1st stimulus is sufficient to elicit firing, the inhibitory recurrent pathway may be activated and thus the field IPSP, reflected in a positive wave, may occur. If the 2nd stimulus is given during this early IPSP the resulting field EPSP has a smaller amplitude. In this interpretation we did not mention the possibility of the stimuli also evoking feed-forward inhibition (Buszaki and Eidelberg 1982). It is likely that such an inhibitory component may contribute to the initial response.

We are now in a position to describe the changes which occurrred in field potentials during kindling. The main changes found were:
- the amplitude of the extracellular EPSP as well as its duration in response to the 1st stimulus increased; particularly the time constant of the decay was prolonged;
- the "late" positive component tended to disappear and was substituted by a potential with negative polarity;
- the ratio of the amplitude of the extracellular EPSP evoked by the 1st and by the 2nd stimulus changed drastically in favor of the 2nd response.

The changes in amplitude of the field EPSP (negative wave) and of the "late" positive component along the different phases of kindling are shown, as function of stimulus intensity in Fig. 2.

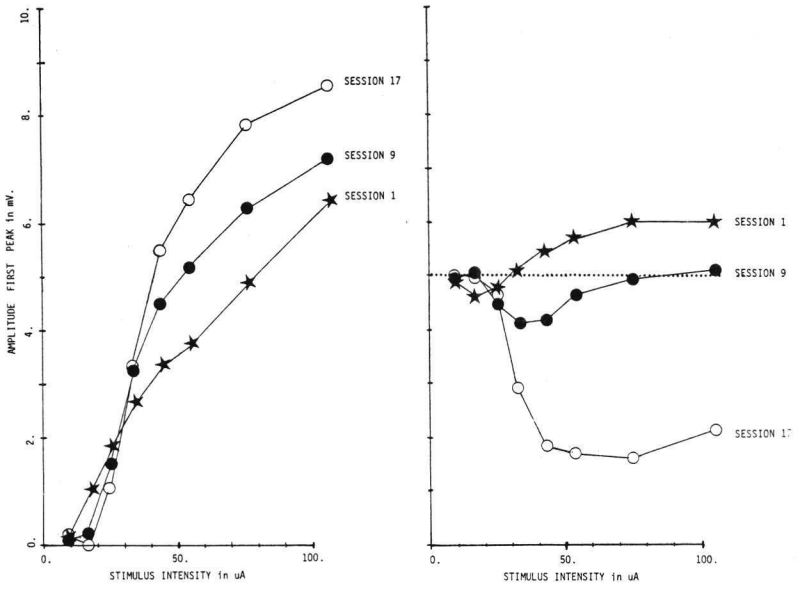

Fig. 2. Plot of amplitude against intensity of the pulse applied to the Schaffer Collaterals (in μA). A: amplitude of the top of the field EPSP; note the increase in amplitude with kindling session. B. Amplitude of late wave (measured at about 17 ms after pulse) which is positive in the control period (session1), becomes alsmost zero at session 9 and clearly negative at session 17.

Discussion

The changes in field potentials evoked by Schaffer collaterals on CA_1 pyramidal cells occurring during kindling may be interpreted as follows:

- the increase in amplitude of the initial extracellular EPSP may be due to an increase in the efficiency of excitatory synapses (for example increased transmitter mobilization and/or a decrease in feed-forward inhibition);
- the conspicuous disappearance of the "late" positive component can be accounted for by a decrease in the feedback inhibition responsible for the initial IPSP;
- the change in the ratio between the 1st and 2nd responses is a consequence of the changes indicated above; the decrease in recurrent inhibition will demask the phenomenon of paired-pulse facilitation which as described above can be put in evidence, in control situations, when the stimulus intensity is low.

A central feature in all these changes is the disinhibition which appears to occur in the neuronal network at the kindling site. We reasoned that if this is indeed a basic process responsible for the change in neuronal excitability during kindling, it should be possible to find a change in the transmitter system responsible for the inhibition. It is known that the initial IPSP is mediated by GABAergic synapses, i.e. synapses which have gamma-aminobutyric acid as transmitter (Knowles et al. 1984). Therefore, terminals should be tested in the hippocampus of kindled rats using specific antibodies for GABA. Preliminary results (Kamphuis et al. 1985) indicated that indeed a decrease in GABA immunoreactivity takes place. This thus may represent an important element in the development of kindling epileptogenesis.
It has also been shown by Wadman et al. (1985) that in hippocampal slices of kindled rats there is a conspicuous change in the dynamics of extracellular CA^{2+}. Slices of kindled animals show in response to a tetanus a significantly larger decrease in extracellular Ca^{2+} than those of controls. This suggests that the cellular elements within a kindled epileptogenic focus present an increased permeability of Ca^{2+}. It is, as yet, not clear how these changes in Ca^{2+} permeability may be related to the decrease in GABA immunoreactivity. An answer tot this question may indeed provide a cornerstone to our understanding of the fundamental cellular processes of epileptogenesis.

Part B
Hippocampal kindling affects spatial memory performances

There is ample evidence, most of it resulting from studies of bilateral lesions, that the hippocampus plays a role in memory tasks involving spatial cues as revealed in radial mazes (Olton et al. 1979, Jarrard 1983). We reasoned that if hippocampal kindling would produce long-lasting deficits in hippocampal functioning, these could be put in evidence by testing rats in such a maze during the proces of epileptogenesis.

Material and Methods

Fourteen male Wistar rats were succesfully implanted as described in part A. The rats were maintained at an average weight of 300 -350 g during the whole experimental period and in a reverse light-dark cyclus of 12:12 h. The behavioral tests were performed in an 8-arm radial maze similar to the one described by Olton and Samuelson (1976); the behavioral procedures followed were as described by those authors. In our case five adjacent arms were baited with food at the beginning of each trial and the other three arms were always empty. The correct response of the rat was to enter each of the five baited arms only once. Deviations from

this response were scored as errors and could be divided into two different classes: 1) if a rat entered an arm for the second time this was scored as a working memory (WM) error; 2) when he entered an unbaited arm for the first time this was scored as a reference memory (RM) error.

If a rat entered a non-baited arm more than once this was scored as a WM error; since it seldom happened in the kindled rat (6 out of a total of 196 trials) and never in controls, scoring these errors as RM instead of WM errors did not affect the conclusions. Comparisons between kindled rats and controls were carried out by using the scores of rats from identical periods of observation. Since no significant differences were found for controls in the various periods, the mean score for controls over the whole time span could equally well have been used; doing so had no influence on the conclusions shown in Table I. The statistical tests used are indicated in the heading of this Table.

TABLE I

Mean error score (mean number of errors per trial) of rats in working (WM) and reference (RM)memory tasks for kindled rats (n=7) and controls (n=7) are shown in the first and last column for period I, II, III. For controls no significant differences among periods were found (means for the whole period WM=0.04, RM=0.68). Differences between periods for kindled rats are shown in column 2 and 3 (Wilcoxon matched pairs sign-ranked test, two tailed probabilities), differences between kindled and control rats for the various periods are given in column 4 (Mann-Whithey U statistic, two tailed probabilities).

mean error score of kindled	kindling periods I	kindling periods II	controls	mean error score of controls
I WM=0.22 RM=1.65			U=44 p<0.01 U=40.5 p<0.05	WM=0.01 RM=0.95
II WM=0.62 RM=1.60	T=3 p<0.05 T=13 ns		U=48.5 p<0.001 U=49 p<0.001	WM=0.06 RM=0.68
III WM=0.18 RM=1.39	T=11 ns T= 8 ns	T=0 p<0.001 T=4 ns	U=29.5 ns U=48 p<0.001	WM=0.11 RM=0.56

Rats were initially trained until a stable performance was obtained; each trial lasted until they had chosen all five baited arms or until a time limit of 15 minutes was reached which however seldomly occurred. After implantation the rats were allowed two weeks for recovery, followed by 25 days of retraining until stable performance was regained.
Rats were randomly assigned to a control and a kindling group of equal size, that were treated identically except for the tetani-

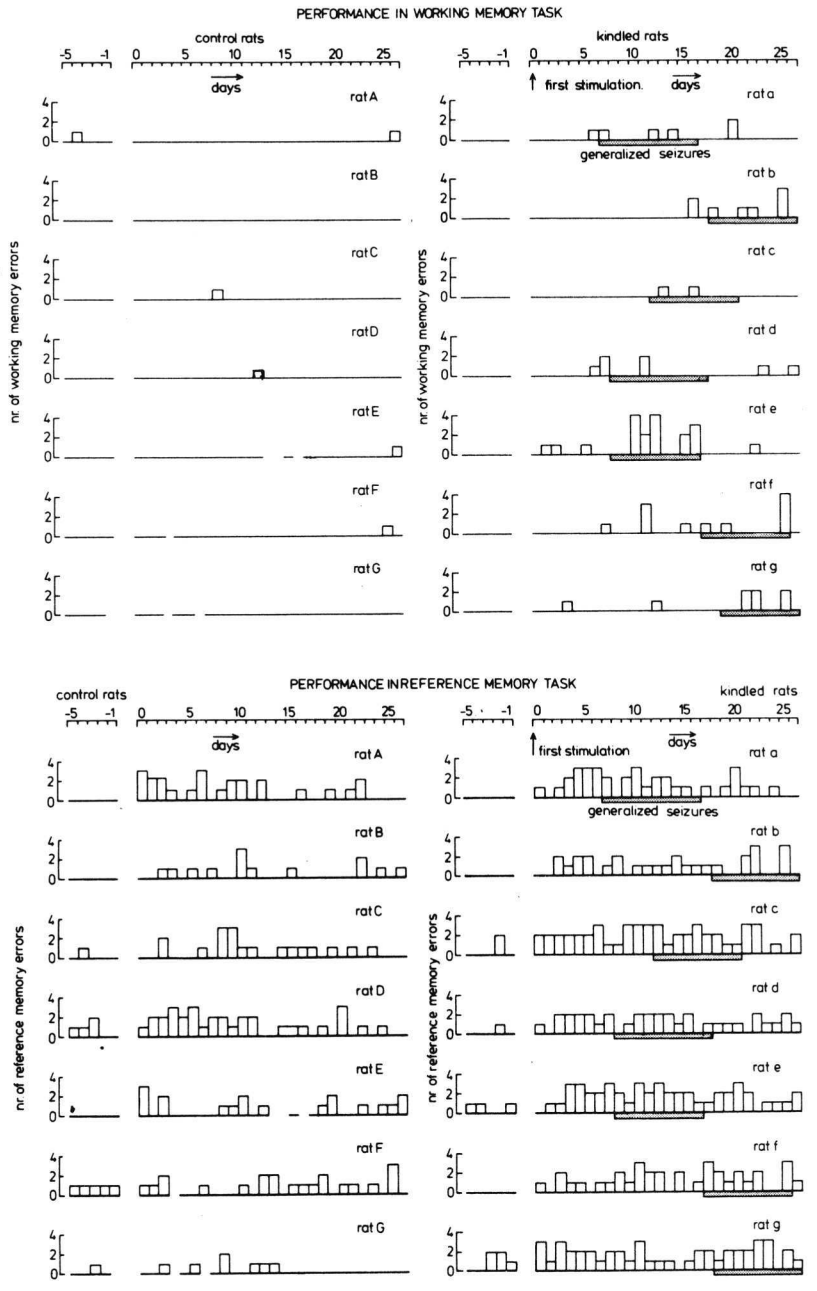

Fig. 3. Distribution along trials (days) of errors made in WM (A) and RM (B) by kindled rats and controls. The period indicated as -5 to 0 is the last part of the training period. The period where the animals showed generalized seizures is indicated by the hatched bar.

zation. Each rat was daily submitted to a behavioral test 1-2 hours after the beginning of the dark period. A tetanus was applied 1-2 hours after this test; in average 5 h later, a second kindling stimulus was administered to reduce the time needed for epileptogenesis. Each stimulus evoked an afterdischarge that lasted between 20 s. in the beginning and 100 s. at the end of the kindling process, which was continued until the first generalized seizure was observed (stage 5 of Racine (1978)). This period varied from rat to rat (range 12-23 days) and will be called period I. From then onwards the tetanus was applied once a day until 9 generalized seizures had been observed (period II); Thereafter the behavioral tests were continued for 7 consecutive days without tetanization to study possible recovery (period III).

Results

The performance of the rats in WM as well as in RM are given as a function of time in fig. 3. Statistical comparison between the two groups and between different periods are summarized in Table I. Error score was calculated as the mean number of errors per daily trial. No significant difference in WM or in RM scores was found between the two groups in the period before kindling started (two tailed Mann-Whitney U-test).

First we will analyse the comparison between kindled animals and controls.
For the RM task, there were significant differences during all periods; these differences increased slightly from period I to II and are still present after kindling was discontinued (III). For the WM task a significant difference between kindled animals and controls was also found during kindling (I, II), but when stimulation was stopped, performance improved and it was not different from controls any more in period III.

Secondly, we will compare the performance of the kindled rats among the three periods.
Comparisons between any two different periods were made by Wilcoxon matched-pairs signed rank test (see Table I). RM did not show significant changes between periods I and II; nor between periods II and III, which implies that there was hardly any recovery. However WM performance significantly degraded from period I to II, but recovered from period II to III, even to such an extent that it was in period III not significantly different from period I.

Discussion

It must be concluded that kindling of the left dorsal hippocampus is sufficient to disrupt the normal functioning of both hippocampi in such a way that a deficit in WM and RM is apparent. Furthermore, we were able to demonstrate that after stopping the kindling stimulation a certain degree of recovery is possible, which was at least significant for WM. In this respect, there appears to be a difference between the defects in WM and RM, the latter lasting longer.

The behavioral deficits seen after left dorsal hippocampal kindling should be compared with behavioral changes produced in a similar or related way. Changes in working memory have only been reported after bilateral disconnection of the hippocampus, by Olton et al. (1979) but a similar impairment in reference memory has been shown recently after kainic acid lesions of the hippocampus, by Jarrard et al. (1983). Ehlers and Koob (1985) showed that hippocampal kindling may cause a decrease in locomotor activity but this was limited to a period of 2 h after a seizure; rats treated with injections of tetanus toxin in the hippocampus (George and Mellanby 1982) showed an amnestic syndrome in a discrimination task, but this treatment dit not cause lasting impairment of the short-term memory.

Each kindling stimulus may produce a certain degree of retrograde amnesia (Olton and Wolf 1981). The time between the test and the tetanus was however never shorter than 45 minutes and lasted usually 1-2 h; the next behavioral test was performed 18-23 h later. Therefore, the findings presented here represent a long lasting disruption of the processes involved in the spatial task that uses reference and working memory. We should, however, stress that the task relies on spatial cues (O'Keefe and Nadel 1978). This implies that we cannot generalize our findings without further explicit evidence to other memory tasks, which do not involve such cues. It seems of particular interest to relate these findings to the clinical observation that epileptic patients with limbic foci may have verbal memory deficits and that impairments in a number of cognitive functions correlate with focal limbic epileptiform activity (Wieser et al. 1985). Summarizing, hippocampus kindling provides not only a model of focal motor seizures but also of behavioral deficits correlated with epileptogenesis.

General Conclusions

The results obtained with the kindling model of epilepsy in the hippocampus of the rat described here, permit the conclusion that

repeated electrical stimulation provokes a long-lasting impairment of hippocampal neuronal networks, which is revealed not only in a number of electrophysiological properties but also in specific behavioral deficits. The electrophysiological changes appear to consist mainly in a decrease of inhibitory processes, probably of the recurrent type. This will lead to a decrease in the stability of the network. We therefore formulate the hypothesis that the kindling process will lead to a decrease of functioning GABA-mediated synapses. This hypothesis is currently being verified using immunocytochemical methods. As mentioned in part A, one main question which remains unsolved is how such changes in GABA inhibition are related to the large increase in Ca^{2++} permeability which was shown to occur in kindled tissue by Wadman et al. (1985). This problem is a matter of future research.

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References

Andersen, P., Eccles, J.C., and Loyning, Y. Pathway of postsynap tic inhibition in the hippocampus. J. Neurophysiol., 1964, 27: 608-619.

Andersen, P., Dingledine, R. Gjerstad, L., Langmoen, I.A. and Mosfeldt Laursen, A. The different responses of hippocampal pyramidal cells tot apllication of GABA. J. Physiol., 1980, 305: 279-296

Buszaki, G. and Eidelberg, E. Convergence of pyramidal cells of the rat hippocampus. Brain Res., 1982, 237: 283-295.

Creager, R., Dunwiddie, T. and Lynch, G. Paired-pulse and fre quency facilitation in the CA_1 region of the in vitro rat hippocampus. J. Physiol., 1980, 229: 409-424.

Ehlers, C.L. and Koob, G.F. Locomotor behaviour following kindling in three different brain sites. Brain Research, 1985, 326: 71-79

George, G. and Mellanby, J. Memory deficits in an experimental hippocampal epileptiform syndrome in rats. Exp. Neurology, 1982, 75: 678-689.

Goddard, G.V. Development of epileptic seizures through brain stimulation at low intensity. Nature (Lond.), 1967, 214: 1020-1021

Jarrard, L.E. Selective hippocampal lesions and behaviour: effects of Kainic acid lesions on performance of place and cue tasks. Behav. Neuroscience, 1983, 97, No. 6: 873-889.

Kamphuis, W., Wadman, W.J. and Lopes da Silva F.H. Loss of gamma-aminobutyric acid (GABA)-immunoreactivity in a kindling induced focus of epileptic activity. Suppl. Neuroscience Letters, 1985 (In Press).

Knowles, W., Douglas, Schneiderman, J.H., Wheal, H.V., Stafstrom, C.E. and Schwartzkroin, Ph.A. Hyperpolarizing potentials in guinea pig hippocampal CA_3 neurons. Cellular and Mol. Neurobiolog., 1984, 3: 207-230.

Leung, L.S. Orthodromic activation of hippocampal CA_1 region of the rat. Brain. Res., 1979, 176: 49-67.

Leung, L.S., Lopes da Silva, F.H. and Wadman, W.J. Spectral characteristics of the hippocampal EEG in the freely moving rat. Electroencepha. clin. Neurophysiol, 1982, 54: 203-219.

Lopes da Silva, F.H., Wadman, W.J., Arnolds, D.E.A.T., Veeken, C. and Holsheimer, J. Hippocampus: behaviour and modulation of local circuits. In: F. Reinoso-Suarez and C. Ajmone-Marsan (Eds) Cortical Integration. Raven Press, New York, 1984: 147-170.

McNamara, J.O., Byrne, M.C., Dashief, R.M. and Fitz, J.G. The kindling model of epilepsy: a review. Progr. in Neurobiol., 1980, 15: 139-159.

O'Keefe, J. and Nadel, L. The hippocampus as a cognitive map. Clarendon Press, Oxford, England, 1978.

Olton, D.S. and Wolf, W.A. Hippocampal seizures produce retrograde amnesia without a temporal gradient when they reset working memory. Behav. and Neurol. Biol., 1981, 33: 437-452.

Olton, D.S. and Samuelson, R.J. Remembrance of placed passed: spatial memory in rats. J. exp. Psych.: An. Behav. Proc., 1976, 2: 97-116.

Olton, D.S., Becker, J.T. and Handelman, G.E. Hippocampus space and memory. Behav. and Brain Sci., 1979, 2: 313-365.

Racine, R. Kindling: the first decade. J. Neurosurg., 1978, 3: 234-252.

Ribak, C.E. Axonterminals of GABAergic chandelier cells are lost at epileptic foci. Brain Res., 1985, 326: 251-260.

Ribak, C.E. and Anderson, L. Ultrastructure of the pyramidal basket cells in the dentate gyrus. J. Comp. Neurol., 1980, 192: 903-916.

Schwartzkroin, P.A. Characteristics of CA_1 neurons recorded intracellularly in the hippocampal in vitro slice preparation. Brain Res., 1975, 85: 423-436.

Storm-Mathisen, J. and Ottersen, O.P. Neurotransmitters in the hippocampal formation. In: F. Reinoso-Suarez and C. Ajmone-Marsan (Eds) Cortical Integration, Raven Press, New York, 1984: 105-130.

Wadman, W.J., Lopes da Silva, F.H. and Leung, L.S. Two types of interictal transients of reversed polarity in rat hippocampus during kindling. Electroencepha. clin. Neurophysiol., 1983, 55: 314-319.

Wadman, W.J., Heinemann, U., Konnerth, A. and Neuhas, S. Hippocampal slices of kindled rats reveal calcium involvement in epileptogenesis. Exp. Brain Res., 1985, 57: 404-407.

Wieser, H.G., Hailemariam, S., Regard, M. and Landis, T. Unilateral limbic epileptic status activity: Stereo EEG, behavioral, and cognitive data. Epilepsia, 1985, 26(1): 19-29.

ELEKTROPHYSIOLOGICAL EFFECTS OF CONVULSANTS IN THE HIPPOCAMPUS IN VITRO

R.A. Voskuyl and H. Albus

There is a continuing and vivid interest in epilepsy research concerning the hippocampus and for good reasons. For example, the hippocampus probably has the lowest threshold of all brain areas for epileptiform activity. This agrees well with a study by Gastaut et al. (1975), reporting that complex partial seizures, involving the hippocampus, were the most frequently occurring seizures. Moreover, hippocampal neurons are well known for their capacity to fire in bursts. Such spontaneous bursts are indistinguishable from the Paroxysmal Depolarization Shift (PDS), that can be recorded intracellularly from neocortical or hippocampal neurons exposed to penicillin. Since the PDS is generally considered the hallmark of epilepsy by many, the hippocampus is an obvious target for epilepsy research.

There is, however, another important reason for the interest in the hippocampus. It is relatively large and has a laminated, orderly structure with well defined inputs and outputs, which is an experimental advantage, both technically and conceptually. Maximal advantage of these porperties is taken by using the in vitro slice preparation of the hippocampus. This area of research has grown explosively in recent years and the reader is referred to monographs by Kerkut and Wheal (1981) and by Dingledine (1984) for recent reviews on this topic.

A simple and convenient method to induce epileptiform activity in the hippocampal slice preparation is by applying convulsant drugs via the perfusion fluid or with a micropipette. The effects of several convulsants have been investigated on the hippocampal slice, in particular penicillin, picrotoxin, bicucullin and to a lesser extent kainic acid and pentylenetetrazole (e.g. Schwartzkroin and Prince 1977; Dingledine and Gjerstad 1980; Westbrook and Lothman 1983; Hablitz 1984). Although the effects of these drugs are not identical and the individual drugs may exert more than one action, it has been proposed (Alger, 1984) that the antagonism to GABA (Dingledine and Gjerstad 1980, Schwartzkroin and Prince 1980) is the key to their convulsant action. Suppression of GABAergic inhibition releases an intrinsic capacity to fire in bursts and leads to spontaneous epileptiform activity. There is, however, at least one group of convulsant drugs that does not seem to obey this rule, i.e., the aminopyridines. Studies on peripheral nervous tissue have shown that 4-aminopyridine (4-AP)blocks 2 types of K^+ currents, the delayed rectifying current (Ulbricht and Wagner 1976), which is involved

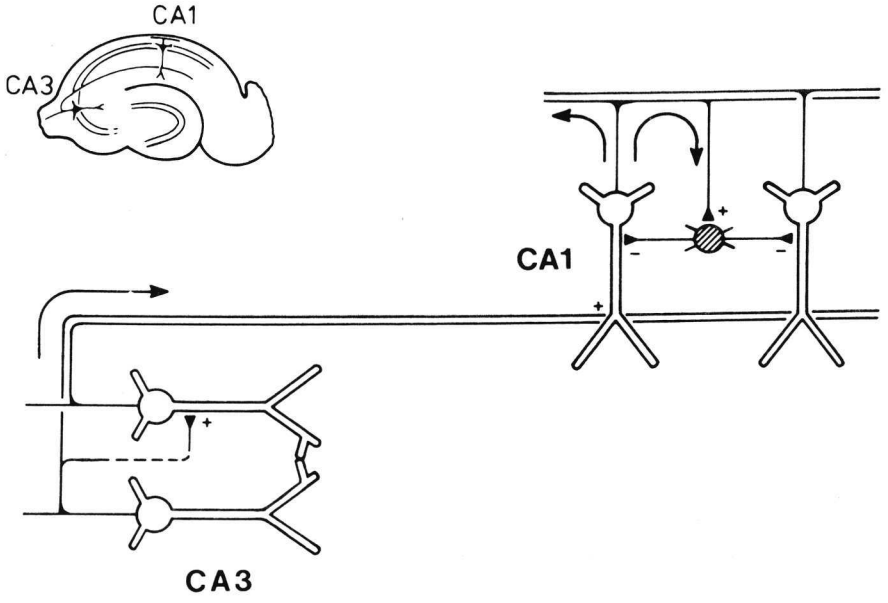

Fig. 1. A. Transverse hippocampal slice. Only the CA1 and CA3 fields, the orientation of the pyramidal cells and the projection from CA3 to CA1 are indicated here. B. Schematic representation of synaptic connections in and between CA1 and CA3. CA3 pyramidal cells project via the Schaffer collaterals to CA1 pyramidal cells making synaptic contacts "en passage". Recurrent excitation in CA3 is represented by an interrupted line, indicating the uncertainty whether or not interneurons are involved. In CA3 also an electrotonic contact is indicated. In CA1 recurrent inhibition via an interneuron is indicated.

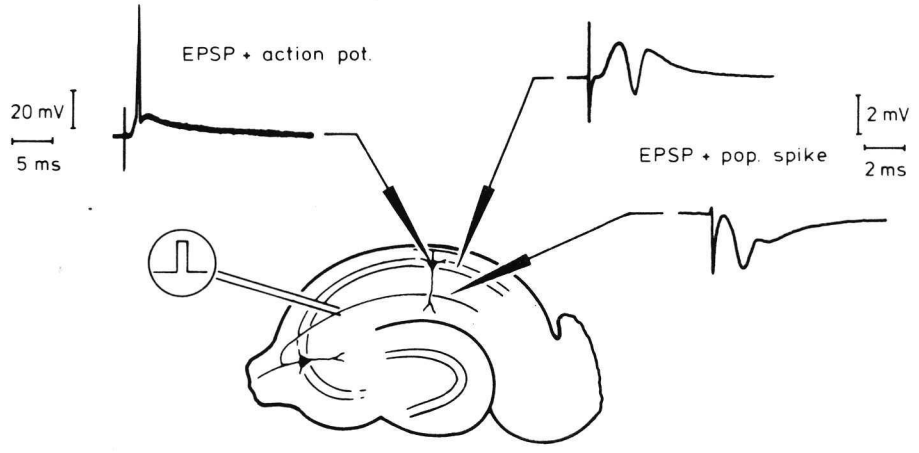

Fig. 2. Examples of intracellular and extracellular responses in CA1 measured after a single stimulus to the Schaffer collateral system. EPSP: excitatory postsynaptic potential. Positivity is upward in this and all subsequent figures.

in termination of the action potential, and a fast transient current (I_A) (Thompson 1982), which possibly plays a role in regulating neuronal excitability. 4-AP also strongly enhances transmitter release, both at excitatory and inhibitory synapses (Thesleff 1980) and this action has also been demonstrated in the hippocampal slice (Buckle and Haas 1982). Therefore, we started a study to investigate the convulsant properties of 4-AP in the hippocampal slice to see whether epileptiform activity induced by a compound that enhances synaptic inhibition differs from the aforementioned convulsants.

The hippocampus is a bilaterally symmetrical structure, shaped somewhat like a cashew nut and consists of two archicortical fields: the hippocampus proper or cornu ammonis and the dentate gyrus. In this study we will be mainly concerned with the CA1 and CA2/CA3 fields in the cornu ammonis. The pyramidal cells comprise the majority of neurons in these fields and these cells are arranged in parallel, their somata forming a continuous sheet. Another important aspect of the structure of the hippocampus is that some of the major connecting pathways run in a plane normal to the longitudinal axis of the hippocampus. In transverse slices these pathways are preserved, for example the Schaffer collateral pathway containing fibers projecting from CA3 pyramidal neurons to CA1 cells, making "en-passage" contacts to the apical dendrites (Fig. 1). There are also local circuits within area CA1 and CA3 (Fig. 1). Basket cells receive an excitatory input from the pyramidal cells and exert an inhibitory influence on the same cells. Recurrent excitatory pathways (Miles and Wong 1983) and electrotonic contacts have also been described.

The procedure for preparing slices is briefly as follows. Wistar rats are anaesthetized with ether, decapitated, whereafter the brain is quickly removed and transferred to chilled ACSF (Artificial Cerchrospinal Fluid). The hippocampi are dissected and placed on moistened filter paper. 400 µm slices are made with a modified McIlwain chopper and transferred to a continuous perfusion incubation chamber.
The ACSF consists of (all values in mM):

Na^+	149	Cl^-	128	glucose	10
K^+	3	SO_4^{--}	1.3	pH	7.4
Ca^+	1.5	$H_2PO_4^-$	1.25		
Mg^{++}	1.3	HCO_3^-	25		

The solution is continuously perfused with 95 % O_2/5 % CO_2; temperature 35° C. 4-AP solutions contained in addition 100 µM 4-AP.

Electrical activity can be evoked in area CA1 by stimulating the Schaffer collateral pathway (fig. 2). With intracellular electro-

des a depolarizing-hyperpolarizing sequence is measured, representing an excitatory and an inhibitory postsynaptic potential (EPSP, IPSP). If the EPSP is strong enough, an action potential is generated. With extracellular electrodes a population EPSP and a superimposed population spike, representing the synchronous activity of pyramidal cells, can be measured.
Due to the parallel arrangement of the pyramidal cells and the location of the synapses at some distance from the somata, the extracellular EPSP is positive in the cell body layer and negative at the level of the synapses. For the population spike the reverse holds true.

When 4-AP was added to the perfusion fluid the first effect was a change in the evoked reponses (Fig. 3). Intracellularly an increase of both the depolarization and the hyperpolarization was observed. Also, the pyramidal cells started firing repetitively to a single stimulus and the latency of the first action potential decreased. Extracellularly the repetitive firing was reflected by the appearance of additional population spikes. An increase in amplitude of the population spikes indicated that more cells were activated by the same stimulus. Upon washout these effects were only slowly reversible.

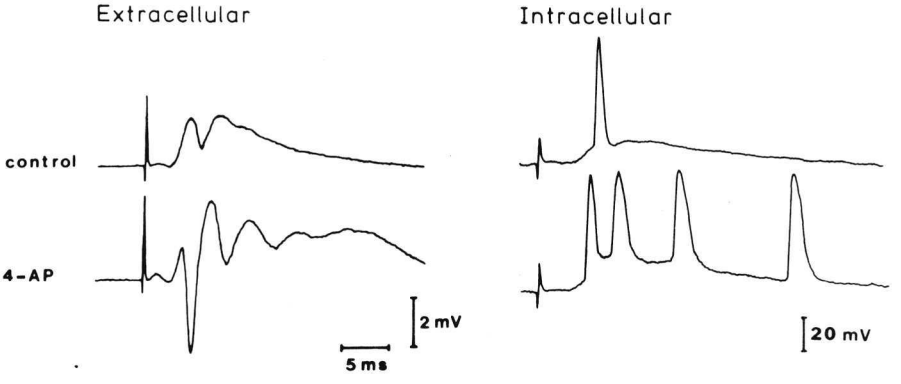

Fig. 3. Changes in intracellular and extracellular evoked reponses after application of 100 µM 4-aminopyridine.

Several minutes after the changes in the evoked potentials, spontaneous field potentials (SFPs) started to appear (Voskuyl and Albus, 1985). This spontaneous activity was seen only when the stimulation rate was low or when there was no stimulation at all, because stimulation tended to suppress spontaneous activity. Spontaneous field potentials usually occurred at regular inter-

vals and had a rather constant amplitude and, once established, this effect remained essentially constant over several hours. The average interval was approximately 1 s. (Fig. 4A). However, in many experiments spontaneous field potentials of a slightly different shape occurred at intervals of about 7 s. (Fig. 4A). These field potentials were more variable in amplitude and duration and in the course of these eperiments it turned out that they represented a different type of spontaneous activity. To differentiate them we have named the SFPs, occurring at intervals of 1 s., type I SFPs and those at 7 s., type II SFPs.

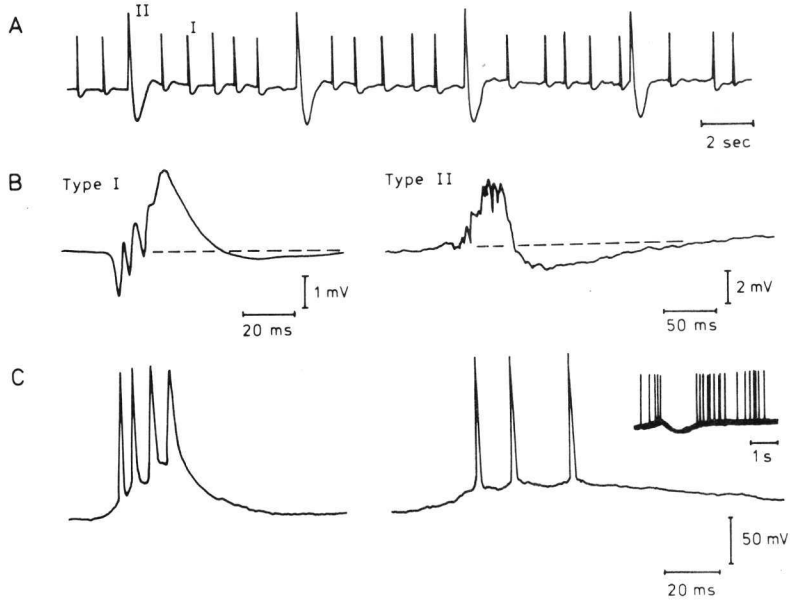

Fig. 4. A. Chart recording of Spontaneous Field Potentials (SFP's) recorded from the soma layer in area CA1. High frequency components are lost in such records due to the low frequency response of the chart recorder. Examples of extracellularly recorded type I and typer II SFPs measures in CA1 in the soma layer (B) and their intracellular equivalents (C).

Measured at the cell body layer type I SFPs consisted of a positive wave followed by a much smaller negative wave (Fig. 4B). In many experiments population spikes were superimposed on the initial slope. Type I SFPs were measured both in area CA1 and CA3 and there were no essential differences in shape. Type II SFPs differed in the sense that they had a more pronounced negative afterwave and that the amplitude in area CA3 was generally rather

small. In occasional experiments type II SFPs had an extremely long duration (up to 1 s.) and were associated with multiple population spikes.
In intracellular recordings there were also differences between the 2 types of spontaneous activity (fig. 4C). Type I SFPs were accompanied by a rapid depolarizing wave associated with a high frequency burst of action potentials and followed by a small hyperpolarization. Type II SFPs were accompanied by a more slowly depolarizing wave and action potentials occurred at a lower frequency and sometimes were altogether absent. They were, however, followed by a pronounced hyperpolarization, which could last up to one second.

When SFPs were measured simulataneously in different areas, it appeared that they occurred nearly synchronously in CA3 and CA1. This raised the question whether spontaneous activity arises simultaneously in all areas of the slice or starts in a specialised region and spreads to other regions. The latter is known to occur after exposure to penicillin or other convulsants. Spontaneous discharges start in a small poplation of cells in the CA3 region, propagate along the Schaffer collateral system and synaptically trigger similar activity in CA1 cells. This was proven by measuring latencies in different regions, transsecting the Schaffer collaterals and pharmacological blockade of synaptic transmission (cf. Schwartzkroin and Prince 1977). We have applied the same strategy in our experiments.

In the first place we measured the latency of occurrence of SFPs in CA1 and CA3 and calculated the propagation speed. SFPs in CA3, both type I and II, always preceded those in CA1. Type I SFPs propagated at a speed of approximately 0.3 m/s., which correlates well with the conduction velocity reported for the Schaffer collaterals (Andersen et al. 1977). On the other hand, type II SFPs travelled 10 times slower and it is, therefore, unlikely that they utilize the same synaptic pathways.
Secondly, we reasoned that if SFPs are triggered synaptically in CA1, the potential profiles along the dendritic axis should resemble the profile of the stimulus evoked EPSP. Such profiles cannot be used as a definitive proof, but are useful as a first approximation. At 50 μm intervals along the dendritic axis SFPs were recorded and averaged and the amplitude was measured at a fixed point in time. Similar to the evoked EPSP (not shown), both type I and type II SFPs were maximally positive at the level of the cell bodies, reversed polarity at about 100-150 μm distance and were maximally negative at about 300 μm distance (Fig. 5).

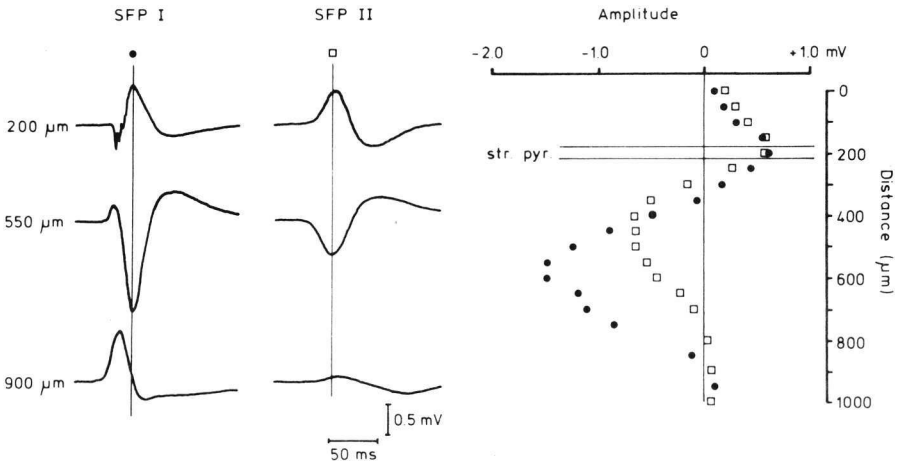

Fig. 5. Samples of type I and type II SFPs measured at 50 µm steps along the dendritic axis in CA1. In the graph the amplitude of the SFPs, measured at a fixed point in time, is shown.

The profiles of type II SFPs were, however, not identical. Reversal point and maximal negativity were closer to the cell body layer. These results suggest that the SFPs recorded in the CA1 region may in part represent excitatory postsynaptic potentials. They also suggest that if this is true, type I and type II SFPs do not propagate along the same synaptic pathways.

In the third series of experiments we transsected the Schaffer collateral pathway, after spontaneous activity had been established. Direction and extent of the cut are indicated in Fig. 6. By stimulating at both sides of the cut and measuring the field potential in CA1, it was verified that the connection between CA3 and CA1 had been completely severed and that the remaining fibres were still functionally intact. Under those conditions type I SFPs continued in CA3, but were completely suppressed in CA1. On the other hand, if the cut included only the Schaffer collaterals as indicated in Fig. 6, type II SFPs were not affected at all. When the cut was extended, the synchrony between CA3 and CA1 was lost, but they were not suppressed in CA1. This turned out to be the ultimate criterion to distinguish between type I and type II SFPs.

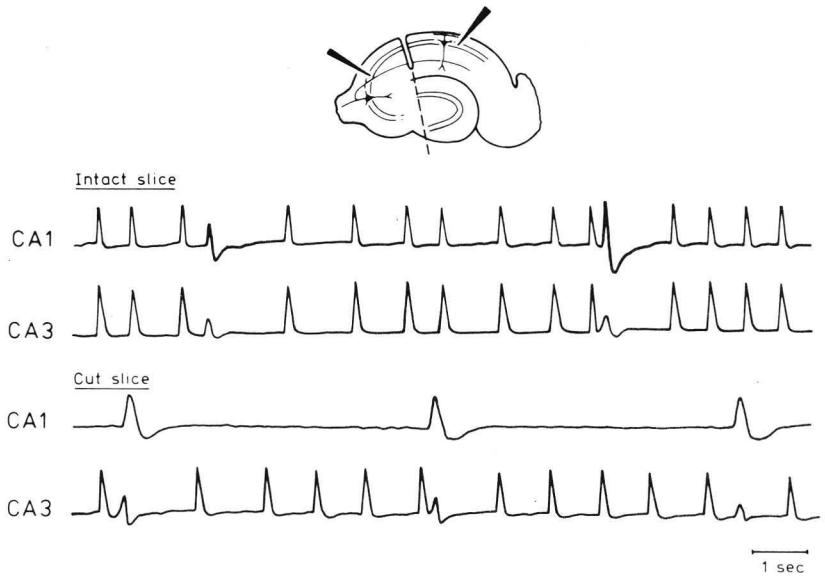

Fig. 6. Simultaneously recorded SFPS in CA3 and CA1 before and after the Schaffer collaterals had been cut. Direction and extent of the cut are indicated in A. If the cut was extended, the synchrony between type II SFPs in CA1 and CA3 was lost (not shown).

Finally we blocked all synaptic transmission by decreasing (CA^{++}) in the perfusion fluid to 0.1 mM and increasing (Mg^{++}) to 5 or 10 mM. This suppresses the evoked EPSP completely with 30 min (Fig. 7A). First, the intervals between type I SFPs increased and subsequently they failed altogether, both in CA3 and CA1. Type I SFPs were suppressed well before the evoked EPSP disappeared completely (Fig. 7B). Upon returning to the original CA^{++} and Mg^{++} concentrations type I SFPs appeared again. Type II SFPs occurred more irregularly but were not blocked by low CA^{++}/high Mg^{++}. Since changing $(Ca^{++})_o$ and $(Mg^{++})_o$ is likely to do more than block synaptic transmission, we checked these results by repeating these experiments with kynurenic acid, a compound which also blocks excitatory synaptic transmission in the Schaffer collateral pathway. The results with kynurenic acid were identical.

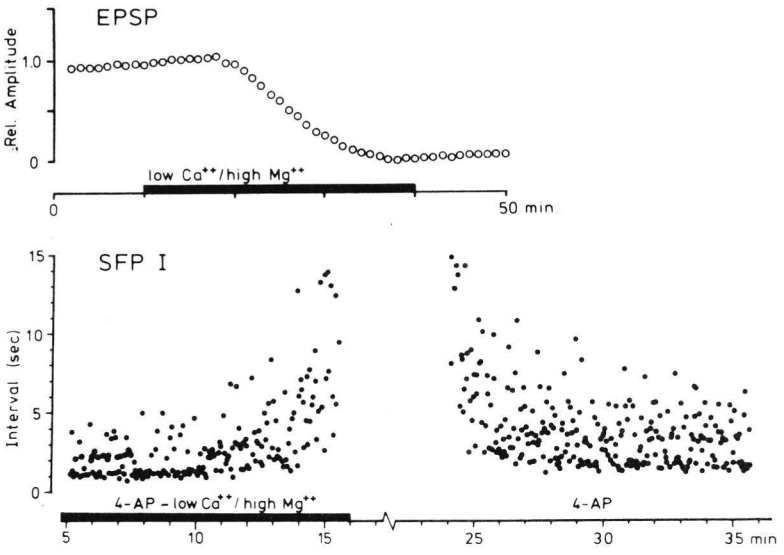

Fig. 7. A. Time course of the suppression of the excitatory postsynaptic field potential by 0.1 mM Ca^{++}/10 mM Mg^{++}. The control value was taken as 100 %.
B. Time course of the effect of low Ca^{++}//high Mg^{++} on the intervals between type I SFPs recorded in the soma layer in CA1.

At present, we attempt to obtain more insight into the mechanisms, which lead to spontaneous activity, by applying the voltage clamp technique. The purpose of the voltage clamp technique is to obtain information on ionic channels (or more precisely ionic conductances) by controlling the membrane potential and measuring the currents required to keep the membrane at that potential. This technique should also allow to discriminate between channels that are activated by changes in membrane potential and channels that are activated by receptor interactions, e.g., a synaptic channel. Since neurons in the hippocampal slice cannot be individually visualised, the conventional voltage clamp technique, using one electrode for measurement of the membrane potential and a second for current injection, cannot be applied. Instead, we used a single electrode voltage clamp circuit which uses the same electrode alternately to measure the membrane potential and to inject current (Fig. 8 A).

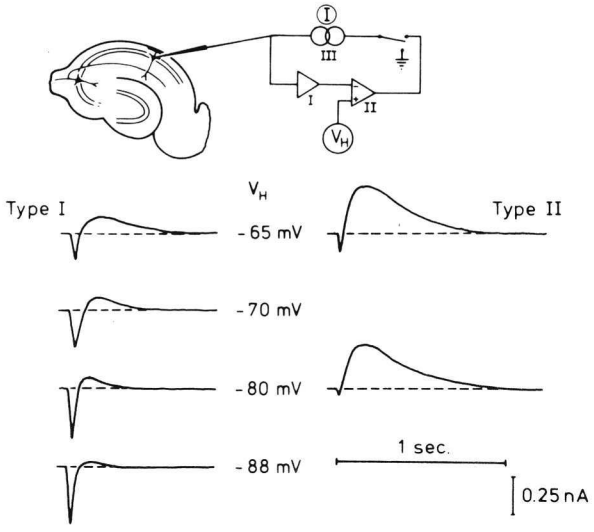

Fig. 8. A. Principle of the "single electrode voltage clamp" circuit. The membrane potential E_M is recorded with a microelectrode and amplified at I, fed into a differential amplifier (II) and compared to the holding potential (V_H) set by the experimenter. If E_M differs from V_H, the output of II causes the current source (III) to inject current via the same electrode to the cell. Since only one electrode is used, measurement of E_M and current injection are alternated.
B. Averaged currents associated with type I and type II SFPs, recorded at different holding potentials (V_H).
Inward (depolarizing) current is downward, outward (hyperpolarizing) current is upward.

Recording simultaneously with an extracellular and an intracellular electrode, it was found that spontaneous field potentials were accompanied by membrane current (fig. 8B). The fact that this was seen at constant membrane potentials suggests that these currents were of synaptic origin. Type I spontaneous membrane currents comprised an inward (depolarizing) and an outward (hyperpolarizing) current. Variation of the membrane potential indicated a reversal potential close to 0 mV for the inward current and more negative than the resting potential for the outward current, which could be consistent with an excitatory and an inhibitory synaptic current respectively. With type II spontaneous membrane currents the inward current was small and variable or absent. The outward current, however, was more pronounced and had a longer duration and could be an inhibitory synaptic current too. These results are still preliminary and more experiments will have to be devised to strenghten these inferences, but at present the simplest explanation is that spontaneous epileptiform activity is associated with synaptic activity.

Taken together, the following picture emerges form these results. 4-Aminopyridine induces two types of epileptiform activity in the hippocampal slice. The first type, occurring about once per s., bears a strong resemblance to spontaneous discharges induced by other convulsants, such as penicillin. It appears that a small

population of neurons in the CA2/CA3 region act as a pacemaker and that epileptiform bursts generated there, spread along synaptic pathways to other areas. The effects of different convulsants seem to differ only quantitatively. This has two important implications. Firstly, the intrinsic properties of the hippocampal neurons and the way they are connected are apparently a more important factor for the generation of epileptiform activity than the specific properties of a certain convulsant. Secondly, the present results indicate that blockade of synaptic inhibition is not strictly necessary to tip the balance. All presently available evidence (Thesleff 1980; Buckle and Haas 1982) including our own intracellular recordings point to an increase of synaptic inhibition by 4-aminopyridine. On the other hand, it still has to be clarified what the key action for 4-aminopyridine is. A possible candidate is, for example, the increase in spontaneous transmitter release, including at excitatory synapses, that is caused by 4-aminopyridine (e.g. Buckle and Haas 1982).

The second form of spontaneous activity, occurring approximately once per 7 s., has not hitherto been reported for other convulsants. Characteristic for this type of activity is the lower frequency of occurrence, the fact that initiation is not limited to a certain region in the slice and the resistance against all forms of synaptic blockade. It was, therefore, somewhat surprising to find that voltage clamp analysis indicated that this type of spontaneous activity is associated with synaptic currents. This may indicate that under normal conditions synaptic activity can contribute to the initiation and propagation of epileptic activity but is not really required. Possibly a disturbance of the regulation of the distribution of ions may play a role in this phenomenon. Presently we are directing our attention to analysis of both types of spontaneous activity with voltage clamp techniques and to the study of the interactions of antiepileptic drugs with these phenomena.

References

Alger, B.E. Hippocampus: Electrophysiological studies of epilep tiform activity in vitro. In: Dingledine, R. (Ed.), Brain slices, Plenum Press, New York 1984: 155-199.

Andersen, P., Sundberg, S.H., Sveen, O. and Wigstrom, H. Specific long-lasting potentiation of synaptic transmission in hippocampal slices, Nature, 1977, 266: 736-737.

Buckle, P.J. and Haas, H.L. Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat, J. Physiol., 1982, 326: 109-122.

Dingledine, R. (Ed.) Brain Slices, Plenum Press, New York, 1984.

Dingledine, R. and Gjerstad, L. Reduced inhibition during epileptiform activity in the in vitro hippocampal slice, J.Physiol., 1980, 305: 297-313.

Gastaut, H., Gastaut, J.L., Goncalves e Silva, G.E. and Fernandez Sanchez, G.R. Relative frequency of different types of epilepsy: a study employing the classification of the International League aginst Epilepsy, Epilepsia, 1975, 16: 457-461.

Hablitz, J.J. Picrotoxin-induced epileptiform activity in hippocampus: role of endogenous versus synaptic factors, J. Neurophysiol., 1984, 51: 1011-1027.

Kerkut, G.A. and Wheal, H.V. (Eds.) Electrophysiology of isolated mammalian CNS preparations, Academic Press, London, 1981.

Miles, R. and Wong, R.K.S. Single neurons can initiate synchronized population discharge in the hippocampus, Nature, 1983, 306: 371-373.

Schwartzkroin, P.A. and Prince, D.A. Penicillin induced epileptiform activity in the hippocampal in vitro preparation, Ann. Neurol., 1977, 1: 463-469.

Schwartzkroin, P.A. and Prince, D.A. Changes in excitatory and inhibitory synaptic potentials leading to epileptogenic activity, Brain Research, 1980, 183: 61-76.

Thesleff, S. Aminopyridines and synaptic transmission, Neuroscience, 1980, 5: 1413-1419.

Thompson, S. Aminopyridine block of transient potassium current, J. Gen. Physiol., 1982, 80: 1-18.

Ulbricht, W. and Wagner, H. H. Block of potassium channels of the nodal membrane by 4-aminopyridine and its partial removal on depolarization, Pflugers Arch., 1976, 367: 77-87.

Voskuyl, R.A. and Albus, H. Spontaneous epileptiform discharges in hippocampal slices induced by 4-aminopyridine, Brain Research, in press.

Westbrook, G.L. and Lothmann, E.W. Cellular and synaptic basis of kainic acid-induced hippocampal epileptiform activity, Brain Ret., 1983, 273:97-109.

SOME BIOCHEMICAL ASPECTS OF RESEARCH IN EPILEPSY: A SPECULATIVE SYNTHESIS

O.R. Hommes and M.G.P. Feenstra

The direction in which biochemical research in epilepsy proceeds is strongly determined by our knowledge of the functioning of the central nervous system (CNS). One of the basic aspects of this functioning is that communication between neurons generally is provided for by neurotransmitters acting on specific receptors. These receptors, located in the membranes of various parts of the neuron from dendrites to axon terminals, are thought to be coupled to functional entities such as ion channels or second messenger generating systems. The receptors located on the dendrites and soma presumably control the activity of the neuron, i.e. whether the neuronal membrane is depolarized and a specific neurotransmitter is released onto other neurons or whether the membrane is hyperpolarized and the release does not take place. The receptors on the axon terminals are thought to modulate the synthesis and release of neurotransmitter. Amongst all these various receptors are also "autoreceptors" by which the neuron may provide its own feedback.

A particular neuron may receive signals from a large number of neurons, differing in their location and in the neurotransmitters they are carrying, which combined actions will determine the activity of this particular neuron, whether it will fire (and release its own neurotransmitter), or whether it will remain silent.

A neurotransmitter may have excitatory actions and will bring a neuron closer to firing when enough of its receptors are activated, or inhibitory actions, and will keep a neuron from firing when enough inhibition-coupled receptors are activated. Basically, epileptic reactions can be evoked when for a group of neurons the physiological balance between excitatory and inhibitory influences is grossly disturbed. This can be done experimentally by potentiating or mimicking the effects of excitatory neurotransmitters or by antagonizing the effects of inhibitory neurotransmitters (Meldrum 1978; Woodbury 1984).

Another basic aspect of CNS functioning is that neurons need a constant and sufficient supply of glucose and oxygen to maintain their activity. The CNS depends on glucose for energy and as carbon source and has a high rate of oxygen consumption for carbohydrate oxidation. It is likely that the high energy demand of the brain compared to other tissues is needed for the continuous

sustainment and restoration of membrane potentials in active neurons. Experimentally induced epilepsy has been shown to increase several indices of metabolic rate (Duffy and Plum 1976; Sokoloff 1976; Ingvar et al. 1984; Evans and Meldrum 1984).

In this short review we will focus on the relation between epilepsy and the two basic aspects of CNS functioning mentioned above, i.e. the balance between excitatory and inhibitory transmitters and the rate of cerebral metabolism.

In epilepsy research an emphasis has always been laid on the measurement of electrophysiological events by electrodes placed on or in the brain or by single cell recordings in the intact brain or in brain tissue slices. These studies have provided a firm basis for further biochemical and pharmacological research. For instance, we have learned that neurons which participate in epileptic activity often show a specific electrophysiological behaviour called the paroxysmal depolarization shift (PDS) (Elger and Wieser 1984). The "epileptic neurons" show during the spikes, as recorded from the brain surface, a rapid and sustained membrane depolarization with a number of high frequency action potentials during the initial phase. These giant postsynaptic potentials have been proposed to be induced in normal neurons by a potent synchronous activity of other normal neurons (Roberts 1984), while others suggest that the PDS is generated by endogenous mechanisms (Elger and Wieser, 1984). However, it appears that these actions both play a role in the initiation and propagation of paroxysmal depolarizing activity (Prince and Connors 1984). Some neurons, possibly with membrane properties that are different from the majority, may function as pacemakers of epileptiform discharges once they are more than normally excited, e.g. when they are disinhibited. With a proper system of neural excitatory connections these pacemakers might synchronize to such an extent that they recruit other groups of neurons. A most important part in this - still hypothetical - process is played by disinhibition, i.e. the decrease of normally occurring inhibition. To understand this we will have to take a closer look at the balance between inhibitory and excitatory actions in the CNS.

Quantitatively, amino acids are the major neurotransmitters in the mammalian CNS, both with regard to concentrations and to number of neurons. In the brain glutamic acid and gamma-aminobutyric acid (GABA) appear to be the major excitatory and inhibitory amino acids, respectively. In the cerebral cortex the vast majority of the neurons contain either glutamate- or GABA-like immunoreactivity (Ottersen and Storm-Mathisen 1984), although this does not mean that all glutamate-containing neurons use this amino acid as a neurotranmitter. While extensive

glutamate-using neuronal pathways originating a.o. in the cerebral cortex and the hippocampus have tentatively been identified, the majority of the GABA-using neurons appear to function in local circuits, e.g. in the cerebral cortex (Fagg and Foster 1983). These GABA-ergic interneurons are of extreme importance in limiting and modulating the excitatory activity in various brain regions. Roberts (1984) has put forward schemes of neural units which show convincingly the importance of the inhibitory interneurons. Part of the neural units involving the cerebral cortex are the cortical modules proposed by Szentágothai (1975) that are characterized by well defined spaces in which inhibitory interneurons are active. Excitation within such a module or column might first affect a pacemaker neuron which would subsequently activate the other efferent parts of the module but inhibit the neighbouring modules. The spreading excitation may be direct but more probably indirect i.e. using disinhibition. The latter process may be thought of as an activation of phasically active inhibitory interneurons terminating on other interneurons which tonically inhibit the efferent pyramidal cells (Roberts 1984). The excitation within a given module would normally be set to boundaries by recurrent and feedback activation of inhibitory interneurons. Thus, both the intramodular spread of excitation and the "recruitment" of other modules into the excitation is limited by the inhibitory activities.

The most important neurotransmitter in these inhibitory processes is likely to be GABA (Roberts 1984; Mandel et al. 1982). GABA that is released will activate the classical bicuculline-sensitive receptors that are coupled to chloride-ion channels that mediate inhibition. Recently, these receptors have been designated GABA-A to separate them from bicuculline-insensitive GABA-B receptors not coupled to chloride ionophores (Bowery et al. 1984). These latter receptors may mediate the GABA-induced reduction of evoked release of various neurotransmitters. With our present state of knowledge it is the GABA-A receptor which is the most relevant to the role of GABA in epilepsy. This receptor is part of an intricate complex of macromolecules situated around the chloride ion channel (Polc et al. 1982; Olsen et al. 1984). Other parts of this complex are the nanomolar benzodiazepine receptor and binding sites for picrotoxine-like convulsants and barbiturates (Trifiletti et al. 1985). Although it is attractive to give this complex the key position in the anticonvulsant effects of benzodiazepines and barbiturates and even of valproic acid (Olsen et al. 1984) and phenytoin (Spero 1982), the evidence for this is not conclusive and a separate binding site for micromolar concentrations of benzodiazepines related to certain anticonvulsant actions of these drugs has been reported (Delorenzo 1984).

While it is too early to decide about the molecular mechanisms of those anticonvulsants whose action has been related to the GABA-ergic system, it should be clear that there is a vast amount of evidence showing that in one way or another many anticonvulsant drugs are able to increase GABA-ergic transmission to anti-convulsant effects (Krogsgaard-Larsen 1981; Lloyd 1983; Fariello and Ticku 1983; Meldrum 1984). Indeed, there are many possible ways to enhance GABA-mediated inhibition in the CNS (Krogsgaard-Larsen 1981; Meldrum 1984) and there may also exist brain regions where a local increase in GABA-ergic transmission results in potent anticonvulsant activity. The evidence that the substantia nigra is such a site is rapidly accumulating (Iadarola and Gale 1982; Le Gal La Salle et al. 1983; McNamara 1984).

In addition to this there is an overwhelming body of evidence that inhibition of GABA-ergic transmission leads to epileptiform reactions (Meldrum 1978; Mandel et al. 1982; Woodbury 1984; Roberts 1984). The reverse is not true as there are various models of epilepsy and various forms of human epilepsy where GABA does not seem to be involved (Fariello and Ticku 1983; Bartholini 1985). However, in focal epilepsy, even in those models where the GABA system does not seem to be directly involved, such as the epilepsy induced by cortical application of alumina or cobalt, the development of the epileptic activity appears to be parallelled by a decrease of parameters of GABA-ergic function (Ross and Craig 1981; Delgado-Escueta 1984). Similar decreases have been reported in human epileptic foci (Delgado-Escueta 1984). How could these decreases develop? Before discussing this, we will first look at the excitatory systems that are inhibited by GABA.

Glutamic acid is a very important excitatory neurotransmitter in various brain regions, e.g. the cerebral cortex and the hippocampus (Fagg and Foster 1983; Fonnum 1984). These brain areas have high densities of glutamate receptors and are the oirigin of glutamate-using neuronal pathways. Indeed, cortical afferent fibres are often glutamatergic (Fagg and Foster 1983; Fonnum 1984). Recently, glutamate binding sites have been subdivided in at least three types, according to their sensitivities to different glutamate agonists (Foster and Fagg 1984). These glutamate agonists have potent excitatory actions when applied to neurons and can elicit epileptiform reactions (Zaczek and Coyle 1982). One of the glutamate analogues, kainic acid, has become a frequently used agent in experimental epilepsy. The involvement of glutamate (and aspartate; these two excitatory amino acids are as yet not very well differentiated in their actions) in epilepsy has been shown clearly by the fact that selective antagonists of one of the subtypes of the glutamate receptor are potent anticon-

vulsants in several models of experimental epilepsy (Meldrum 1984).

However, the excitatory glutamate analogues have another very intriguing property, in that they are neurotoxic. Both endogenous substances, such as glutamate itself, various analogues and unrelated normal brain constituents like quinolinic acid and folate derivatives and also amino acids which have not been detected in the mammalian CNS have distinct and sometimes very potent neurodegenerative effects (Olney 1971; Zaczek and Coyle 1982; McGeer et al. 1983; Schwarcz et al. 1984). A simple relationship between epileptogenic and neurotoxic potencies could not be found, suggesting that these effects are mediated by different mechanisms (Zaczek and Coyle 1982). Kainic acid has become a widely used agent to study the relation between experimental seizures and brain damage and to produce localized intracerebral lesions (McGeer and McGeer 1982; Coyle 1983). Its mechanism of action is still a matter of debate (Ferkany et al. 1984), whether it involves a direct neurotoxic effect or an indirect one by stimulating glutamate release. In addition effects secondary to the sustained paroxysmal excitation (edema and massive ionic imbalance) may be relevant (Lassmann et al. 1984). In vitro studies suggest differential activation of ion channels by different excitatory amino acids (Mat Jais et al. 1984) and it might be speculated that opening of calcium channels would lead to toxic intraneuronal calcium concentration and cell death. Still other mechanisms have been proposed by Rothman (1984) who showed that cell death induced by anoxia may be mediated by the release of glutamate. Inhibition of glutamate re-uptake because of metabolic exhaustion would be sufficient to initiate this process.

If we consider the data referred to in the section on GABA and glutamate several points can be made:

1. GABA-ergic systems provide physiological limits to the propagation and spread of excitatory signals. In experimental epilepsy excitation is taken beyond these limits.
2. In several forms of focal epilepsy there appears to be a direct relation between impairment of GABA-ergic inhibition and epileptiform activity.
3. Breakdown of GABA-ergic inhibition will result in the release of paroxysmal activity of excitatory systems, whose neurotransmitters are known to mediate neuronal degeneration in certain circumstances.
4. Prolonged paroxysmal excitatory activity may cause disturbances in the basic homeostatic systems in the CNS, e.g. membrane alterations, ionic imbalance, edema, metabolic dysfunction or exhaustion, ischemia and hypoxia. Some of these disturbances may directly cause neuronal degeneration, in particular of

presumably sensitive inhibition-mediating interneurons (Roberts 1984). Disturbances could otherwise lead to the release of normal intracellular substances which may act as excitotoxins outside the cell (Schwarcz et al. 1984).

5. Traumatic or other brain lesions may primarily cause gliosis, blood-brain barrier changes or other aspecific alterations and subsequently, in one of the above mentioned ways may impair inhibition.

Of course, point 4 and 5 express thoughts which are at the moment highly speculative. It is not our intention to provide here a comprehensive account of all the data that support the above speculations. Also, we have not taken into consideration the plasticity of the nervous system which may lead to the restoration of function after lesions, nor the involvement of other neurotransmitters and neuromodulators than the ones mentioned above (Meldrum 1978; Delgado-Escueta 1984). However, the basic idea that the disturbed balance between excitatory and inhibitory amino acid neurotransmitters is the first step in epileptogenesis may prove worthwhile in planning future epilepsy research. Indeed, other contributions in this volume provide some very interesting data from our point of view, e.g. the GABA-depletion in the hippocampus during hippocampal kindling (Lopes da Silva 1986) and the membrane alterations in the same model reported by Schrama et al. (1986)

The second basic aspect of CNS functioning that we will discuss in relation to epilepsy is the rate of cerebral metabolism. Changes in several parameters related to the cerebral metabolic rate have been reported during seizures (Duffy and Plum 1976; Sokoloff 1976; Ingvar et al. 1984; Evans and Meldrum 1984). The recent introduction of the labeled deoxyglucose technique coupled to high resolution autoradiography in experimental animals and to non-invasive Positron Emission Tomography (PET) scanning in humans has led to a dramatic increase of research in this field (Ann. Neurol. 1984). However, the technique is still subject to criticisms regarding its basic hypothesis, i.e. that deoxyglucose phosphorylation reflects glucose phosphorylation not only in the normal state but also in states of altered brain activity like epilepsy (Van den Berg and Bruntink 1983; Agranoff and Frey 1984; Cunningham and Cremer 1985). Therefore, the results of both animal and human studies should be regarded with caution. Moreover, during the experiment an averaging effect will occur, while the PET studies are hampered by the limited spatial resolution (Theodore et al. 1983). Because of this, the longlasting interictal periods can be more reliably studied than the relatively short ictal phases.

During the interictal phase in human partial epilepsy interesting results have been obtained (Theodore et al. 1983; Ann. Neurol. 1984). A region of decreased deoxyglucose phosphorylation was found in patients with partial seizures. However, during ictal phases an increased deoxyglucose phosphorylation in the very same region was visible. In several patients this region coincided with electroencephalographically identified foci, while on surgical resection pathological changes were generally found. When combined, these data suggest that in interictal phases of partial epilepsy a region with metabolic changes exists surrounding the focus. These metabolic changes are interpreted as a state of hypometabolism. While aspecific structural and functional changes in and around a lesion may cause this state of altered metabolism, another, more specific, explanation is also possible, although highly speculative.

In the section on GABA we mentioned the organization of the cortex in columns. Activated columns would induce inhibition in the surrounding columns. This was based on electrophysiological measurements. Could it be that this surrounding inhibitory region corresponds to the areas in the human PET scans where the changes interpreted as hypometabolic were found? Collins (1978) observed a "depression of glucose utilization" in cortical regions nearby a focus induced by penicillin injection into the rat cerebral cortex. If such a depression would reflect the increased activity of inhibitory GABA-ergic interneurons one would expect that GABA receptor agonists produce similar alterations in the deoxyglucose model. This has indeed been reported (Kelly and McCulloch 1982). Future studies will learn if the findings in the deoxyglucose model can be substantiated and if the findings in human partial epilepsy using this model bear any relation to the inhibitory areas known from electrophysiological studies.

In this short review we have tried to integrate data from experimental and clinical studies. We have suggested that the basic event in, at least, some forms of partial epilepsy is the highly localized disturbance of the inhibitory limits set to excitatory systems. We have tried to find a relation between these neurotransmitter effects and the suggested hypometabolic regions found in human PET scans. We think that future research in epilepsy should focus on identification of mechanisms that trigger these disturbances. In this respect the studies regarding the structural and functional changes induced by the kindling model of epilepsy (see other papers in this volume) and our own research on the mechanism of action and the effects of endogenous neurotoxic substances such as the folate derivatives appear to be highly relevant. Hopefully, the research on experimental epilepsy in The Netherlands will give us a better insight in the basic

processes underlying epilepsy and will provide us with new directions for clinical studies and future treatment of this disease.

References

Agranoff, B.W., Frey, K.A. A regional metabolic contrast method for the study of brain pathology. Ann. Neurol., 1984, suppl. 15: 93-97.

Bartholini, G. GABA receptor agonists: Pharmacological spectrum and therapeutic actions. Medicinal Res. Rev., 1985, 5: 5-75.

Berg, C.J. van den, Bruntink, R. Glucose oxidation in the brain during seizures: experiments with labeled glucose and deoxyglucose. In: L. Hertz, E. Kvamme, E.G. McGeer, A. Schousboe (eds.), Glutamine, glutamate and GABA in the central nervous system. A.R. Liss, New York, 1983: 619-624.

Bowery, N.G., Price, G.W., Hudson, A.L., Hill, D.R., Wilkin, G.P., Turnbull, M.J. GABA-receptor multiplicity. Visualization of different receptor types in mammalian CNS. Neuropharmacology, 1984, 23: 219-231.

Collins, R.C. Use of cortical circuits during focal penicillin seizures: an autroradiographic study with (^{14}C)deoxyglucose. Brain Res., 1978, 150: 487-501.

Coyle, J.T. Neurotoxic action of kainic acid. J. Neurochem., 1983, 41: 1-11.

Cunningham, V.J., Cremer, J.E. Current assumptions behind the use of PET scanning for measuring glucose utilization in brain. Trends Neurosci., 1985, 8: 96-99.

Delgado-Escueta, A.V. Summation of the workshops and discussion: the new wave of research in the epilepsies. Ann. Neurol, 1984, suppl. 16: 145-158.

DeLorenzo, R.J. Calmodulin systems in neuronal excitability: a molecular approach to epilepsy. Ann. Neurol., 1984, suppl. 16: 104-114.

Duffy, T.E., Plum, F. Seizures and comatose states. In: G.J. Siegel, R.W. Albers, R. Katzman, B.W. Agranoff (Eds), Basic neurochemistry. Little, Brown and Co., Boston, 1976: 388-413.

Elger, C.E., Wieser, H.G. Pathophysiologie der Epilepsie. Schweiz. Med. Wochenschr., 1984, 114: 1278-1288.

Evans, M.C., Meldrum, B.S. Regional brain glucose metabolism in chemically-induced seizures in the rat. Brain Res., 1984, 297: 235-245.

Fagg, G.E., Foster, A.C. Amino acid neurotransmitters and their pathways in the mammalian central nervous system. Neuroscience, 1983, 9: 701-719.

Fariello, R.G., Ticku, M.K. The perspective of GABA replenishment therapy in the epilepsies: a critical evaluation of hopes and concerns. Life Sci., 1983, 33: 1629-1640.

Ferkany, J.W., Zaczek, R., Coyle, J.T. The mechanism of kainic acid neurotoxicity/J. Garthwaite, G. Garthwaite, Reply, Nature, 1984, 308: 561-562.

Fonnum, F. Glutamate: a neurotransmitter in mammalian brain. J. Neurochem., 1984, 42: 1-11.
Foster, A.C., Fagg, G.E. Acidic amino acid binding sites in mammalian neural membranes: their charcteristics and relationship to synaptic receptors. Brain. Res. Rev., 1984, 7: 103-164.
Iadarola, M.J., Gale, K. Substantia nigra: Site anticonvulsant activity mediated by gamma-aminobutyric acid. Science, 1982, 218: 1237-1240.
Ingvar, M. Söderfeldt, B., Folbergrova, J. Kalimo, H., Olsson Y. Siesjö, B.K. Metabolic, circulatory and structural alterations in the rat brain induced by sustained pentylenetetrazole seizures. Epilepsia, 1984, 25: 191-204.
Kelly, P.A.T., McCulloch, J., Effects of the putative GABA-ergic agonists, muscimol and THIP, upon local cerebral glucose utilisation. J. Neurochem. 1982, 39: 613-624.
Krogsgaard-Larsen, P. Gamma-aminobutyric acid agonists, antago nists and uptake inhibitors. Design and therapeutic aspects. J. Med. Chem., 1981, 24: 1377-1383.
Lassmann, H., Petsche, U., Kitz, K., Baran, H., Sperk, G., Seitelberge, F., Hornkiewicz O. The role of brain edema in epileptic brain damage induced by systemic kainic acid injection. Neuroscience, 1984, 13: 691-704.
Le Gal La Salle, G., Kaijima, M., Feldblum, S. Aborative amygdaloid kindled seizures following microinjection of gamma-vinyl-GABA in the vicinity of the substantia nigra. Neurosci. Letters, 1983, 36: 69-74.
Lloyd K.G. Role of GABA-ergic systems in the mechanism of action of anti-epileptic drugs. Thérapie, 1983, 38: 355-362.
Lopes da Silva, F.H., The kindling model of epilepsy of the hippocampus in the rat: electrophysiology and behaviour, Symposium 1985.
Mandel, P., Simler, S. Ciesielski, L. Epilepsy: basic biochemistry and pharmacology. In: M. Sandler, Psychopharmacology of anticonvulsants. Oxford University Press, Oxford, 1982: 1-15.
McGeer, P.L., McGeer, E.G. Kainic acid: the neurotoxic breakthrough. CRC Crit. Rev. Toxicol., 1982, 10: 1-26.
McGeer, P.L., McGeer, E.G., Nagai, T. GABA-ergic and cholinergic indices in various regions of rat brain after intracerebral injections of folic acid. Brain Res., 1983, 260: 107-116.
McNamara, J.O. Kindling: an animal model of complex partial epilepsy. Ann. Neurol., 1984, suppl. 16: 72-76.
Mat Jais, A.M., Kerkut, G.A., Walker, R.J. The ionic mechanisms associated with the excitatory response of kainate, L-glutamate, Quisqualate, ibotenate, AMPA and methyltetrahydrofolate on leech Retzius cell. Comp. Biochem. Physiol., 1984, 77C: 115-126.
Meldrum, B. Amino acid neurotransmitters and new approaches to anticonvulsant drug action. Epilepsia, 1984, 25: 140-149.

Meldrum, B. Neurotransmitters and epilepsy. In: N.J. Legg (ed.), Neurotransmitter systems and their clinical disorders. Academic Press, London, 1978: 167-181.
Olney, J.W. Glutamate-induced neuronal necrosis in the infant mouse hypothalamus. J. Neuropathol. Exp. Neurol., 1971, 30: 75-90.
Olsen, R.W., Snowman, A.M., Lee, R., Lomax, P. Wamsley, J.K. A role of the gamma-aminobutyric acid receptor-ionophore complex in seizure disorders. Ann. Neurol., 1984, suppl 16: 90-97.
Ottersen, O.P., Storm-Mathisen, J. Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. J. Comp. Neurol., 1984, 229: 374-392.
Polc, P., Bonetti, E.P., Schaffner, R., Haefely, W. A three-state model of the benzodiazepine receptor explains the interaction between the benzodiazepine antagonist Ro 15-1788, benzodiazepine tranquillizers, beta-carbolines and phenobarbitone. Naunyn-Schmiedeberg's Arch. Pharmacol., 1982, 321: 260-264.
Prince, D.A., Connors, B.W. Mechanisms of epileptogenesis in cortical structures. Ann. Neurol., 1984, suppl. 16: 59-64.
Research issues in positron emission tomography. Ann. Neurol., 1984, suppl. 15: 1-204.
Roberts, E. GABA-related phenomena, models of nervous system function and seizures. Ann Neurol., 1984, suppl. 16: 77- 89.
Ross, S.M., Craig, C.R. Gamma-aminobutyric acid concentration, L-glutamate-1-decarboxylase activity and properties of the gamma-aminobutyric acid postsynaptic receptor in cobalt epilepsy in the rat. J. Neurosci., 1981, 1: 1388-1396.
Rothman, S. Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. J. Neurosci., 1984, 4: 1884-1891.
Schrama, L.H., Graan, P.N.E. de, Lopes da Silva, F.H., Wadman, W.J., Gispen, W.H. A possible role of hippocampal phosphoproteins in epileptogenesis
Schwarcz, R., Foster, A.C., Franch, E.D., Whetsell W.O., Köhler, C. Excitotoxic models for neurodegenerative disorders. Life Sci., 1984, 35: 19-32.
Sokoloff, L. Circulation and energy metbolism of the brain. In: G.J. Siegel, R.W. Albers, R. Katzman, B.W. Agranoff (Eds), Basic neurochemistry. Little, Brown and Co., Boston, 1976: 388-413.
Spero, L. Neurotransmitters and CNS disease. Epilepsy. Lancet II 1982: 1319-1322.
Szentágothai, J. The "module-concept" in cerebral cortex architecture. Brain Res., 1975, 95: 475-496.

Theodore, W.H. et al. (^{18}F)Fluorodeoxyglucose positron emission tomography in refractory complex partial seizures. Ann. Neurol., 1983, 14: 429-437.

Trifiletti, R.R., Snowman, A.M., Snyder, S.H. Barbiturate recognition site on the GABA/benzodiazepine receptor complex is distinct from the picrotoxinin/TBPS recognition site. Europ. J. Pharmacol., 1985,106: 441-447.

Woodbury, D.M. Neurotransmitters and epilepsy: distinguishing characteristics and unifying precepts. Fed. Proc., 1984, 43: 2529-2531.

Zaczek, R., Coyle, J.T. Excitatory amino acid analogues: neurotoxicity and seizures. Neurpharmacol, 1982, 21: 15-26.

PREDICTION OF OUTCOME OF ANTIEPILEPTIC DRUG WITHDRAWAL

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When a patient who has previously had epilepsy is seizure-free for several years on antiepileptic drugs (AEDs), the question of withdrawing medication arises. There are discrepancies in the

TABLE I

Derivation of study population

Patients attending 2 epilepsy clinics		1485
3 yr seizure-free and history of at least 3 undoubted epileptic seizures		221
Additional patients as above from 3rd clinic		6
		227
Less:- Not taking AEDS in effective dose	27	
Outside age range 18-60 yr	45	
IQ below 70	20	
Neurological deficit	8	
Psychiatric disorder	8	
Pregnancy or somatic disease	2	
Relapsed before entry	6	116
Eligable to enter study		111
Less:- Unwilling to participate		32
Entered and initial EEG performed		79
Less:- Not seizure-free during initial EEG	9	
Relapsed before AED reduction	4	
Withdrew consent	4	17
Completed study		62

figures published both for the probability of becoming seizure-free on medication and for avoiding relapse on withdrawal. Population studies suggest 70 to 80% of new patients are seizure-free within 8 years and that only 20% relapse on subsequent withdrawal. In epileptological practice, however, only 30 to 40% of patients become seizure-free and 60% or more of adult patients relapse when drugs are withdrawn. Nevertheless, increasing awareness of the risks of long term AED administration and of the impairment of cognitive function produced in many patients by

TABLE II

AED withdrawal schedule showing 2 weekly steps from initial dose

	WITHDRAWAL STEPS (2 wk intervals)								
PB	300	250	200	150	100	75	50	25	PB
MPB	300	250	200	150	100	75	50	25	MPB
PRM		1500	1250	1000	750	500	250	125	PRM
PHT	400	350	300	250	200	150	100	50	PHT
ESM			1500	1250	1000	750	500	250	ESM
MSM						900	600	300	MSM
AZA						750	500	250	AZA
STA						600	400	200	STA
CBZ			1200	1000	800	600	400	200	CBZ
VPA			1800	1500	1200	900	600	300	VPA
CZP	4	3	2	1.5	1	0.75	0.5	0.25	CZP
DZP				25	20	15	10	5	DZP
NZP				25	20	15	10	5	NZP

Legend:

PB : phenobarbitone (Luminal)
MPB: mephobarbital (Prominal)
PRM: primidone (Mysoline)
PHT: phenytoin (Difantoïne)
ESM: ethosuximide (Zarontin
MSM: methsuximide (Celontin)
AZA: acetazolamide (Diamox)
STA: sulthiam (Ospilot)
CBZ: carbamazepine (Tegretol)
VPA: valproate (Depakine)
CZP: clonazepam (Rivotil)
DZP: diazepam (valium
NZP: nitrazepam (Mogadon)

these drugs encourages doctors and patients to undertake AED withdrawal. It would be valuable to be able to identify patients with a high risk of relapse. There is no consensus on clinical or laboratory findings predictive of relapse in adults. Many authors consider it self-evident that epileptiform EEG activity implies an adverse prognosis, do not attempt AED withdrawal in patients with abnormal EEGs and resume medication if follow-up EEGs show epileptiform discharges. Consequently the belief that an abnormal EEG is predictive of relapse has become a selffulfilling prophesy.

We have recently undertaken a prospective study of AED withdrawal in adults attending specialized epilepsy clinics. A history of at least three undoubted epileptic seizures was required and the patients had been seizure-free for at least 3 years. The study was based on two policlinics of the "Instituut voor Epilepsiebestrijding" with a registered clientele of 1485 patients. As these are specialized epileptological clinics the patients population is heavily weighted with subjects presenting special problems including resistance to therapy. Two hundred and twenty-one patients were nevertheless found who had been seizure-free for 3 yr and had in the past suffered at least 3 undoubted epileptic seizures, excluding febrile or neonatal convulsions and

"Gelegenheitsanfälle". Children were excluded as the prognostic factors in these are known to be different from those in adults, and the elderly as they often prefer to continue the medication to which they are accustomed. Patients with evidence of severe brain damage (neurological deficit or mental subnormality) were also excluded, as this group is known to have a poor prognosis when AEDs are withdrawn. From a third clinic another 6 patients were recruited meeting the above mentioned criteria. Of 111 patients eligible for entry to the study, 32 were unwilling to participate, often for fear of losing their driving licences. Four patients relapsed before AED withdrawal could be commenced, four withdrew their consent and 9 proved during telemetric EEG investigation prior to the study in fact to be having seizures. There remained 62 patients of whom 41, or 66%, relapsed.

AED withdrawal followed a standardized schedule; drugs of third choice were first withdrawn, then those of second choice (ESM, PB, PRM) and the major AEDs (PHT, CBZ, VPA) last.

Where a patient was receiving two drugs of first choice the order of withdrawal was determined by the probable efficacy in the type of epilepsy in question. Thus in patients with partial seizures valproate was withdrawn before phenytoin or carbamazepine, whereas in generalized seizures valproate was the last to be withdrawn. In patients taking both phenytoin and carbamazepine, phenytoin was withdrawn first.
In most cases the withdrawal procedure required from 3 to 6 months.

TABLE III

Timing of relapse in relation to phase of AED withdrawal

% AED REDUCTION	relapses N	cumulative all patients (N = 62)	relapse relapse group (N = 41)
25	1	2%	2%
50	6	11%	7%
75	16	37%	56%
100	5	45%	68%
AEDs WITHDRAWN			
0 - 3 mnth	5	53%	80%
3 - 12 mnth	5	61%	93%
1 - 2 yr	2	65%	98%
> 2 yr	1	66%	100%

Table III shows that the overall chance of remaining seizure-free was 89% during the first withdrawal phase (up to 50% reduction of

AEDs), 55% during the total period of gradual drug discontinuation and 47% within 3 months afther stopping all medication. Two thirds of the recurrences were encountered during withdrawal and 80% within 3 months after stopping all medication. Thereafter the risk of recurrence was slight. Only 1 patient has relapsed more than two years (in fact 26½ month) after stopping all medication. The period of follow up after stopping AEDs ranges from 4 to 6 years.

EEG investigation including a sleep recording and 5 hours telemetry was performed on full medication (M100), when the midpoint of AED withdrawal had been reached (M50), on the completion of AED withdrawl (M0) and at follow-up 4 months later (F4).

Control EEGs during withdrawal comprised 30 min waking records including hyperventilation and photic stimulation. When apparently subclinical epileptiform discharges were observed in the waking state additional measures were adopted to detect possible clinical ictal manifestations, notably recording with the patient sitting erect and with the arms outstretched.

TABLE IV

Semi-quantitative EEG rating scale

EEG findings	+	++	+++
Paroxysmal discharges (Sp.Shw.Spw)	1 - 10	11 - 50	> 50
θ or δ waves (focal or nonfocal)	Present up to 20% of the time	Present 21-50% of the time	Present > 50% of the time

Overall rating:	
Normal	
Midldly abnormal	1. Sligh slowing or irregular rhythmic activity and/or 2. Parosysmal discharges (+), and/or 3. Diffuse θ (+/++) or δ (+), and/or 4. (+/++) Focal θ No focal delta activity permitted.
Moderately abnormal	Any of the above, plus not more than one of the three features listed below.
Severely abnormal	At least two of the three features: 1. Marked disorganization or absence of posterior rhythmic activity 2. (++/+++) δ focal or generalized 3. Paroxysmal discharges (++/+++)

The EEGs were screened for technical quality and for findings requiring urgent action (such as evidence of progressive cerebral pathology) by one of the authors (CDB) who himself had no contact with the patients. The records were subsequently code-numbered, randomized and interpreted independently by two other observers (JAR and JO), using standardized rating scales (Table IV).

TABLE V

Rating system EEG's

Prognosis of Epilepsy - SAERA

Patient _____ EEG no ___
Awake ☐
Sleep ☐ Stage I ☐ II–III ☐ IV ☐
H.V. ☐
P.S. ☐ Rater _____

Posterior Dom. Rythm
Freq. _____ Absent. ☐
Sym. ☐
Asym ☐: ↓on R ☐ ↓on L ☐
Org. ☐ Good ☐ Fair ☐ Poor

Rapid Activity (β)
Sym. ☐
Asym. ☐: ↓R ☐ ↑R ☐ ↓L ☐ ↑L ☐
Excessive ☐

Intermediate Slow (φ)
+ ++ +++
Diffuse ☐ ☐ ☐ R>L ☐
Focal ☐ ☐ ☐ L>R ☐
Location(s)
F ☐ C ☐ P ☐
T ☐ O ☐
R ☐ L ☐

Delta (δ)
+ ++ +++
Diffuse ☐ ☐ ☐ R>L ☐
Focal ☐ ☐ ☐ L>R ☐
Location(s)
F ☐ C ☐ P ☐
T ☐ O ☐
R ☐ L ☐

Spikes
Synch. ☐ Polyspikes ☐
Focal ☐
Location(s)
F ☐ C ☐ P ☐ T ☐
O ☐ R ☐ L ☐
no: 0-10 ☐ 10-50 ☐ >50 ☐
If multifocal state most ac. focus: _____

Sharp Waves
Synch. ☐
Focal ☐
Location(s) F ☐ C ☐ P ☐ T ☐ O ☐ R ☐ L ☐
no: 0-10 ☐ 10-50 ☐ >50 ☐

Spike/Wave Complexes
Synch. ☐ Fr. <2½ ☐ 2½-3½ ☐ >3½ ☐
Focal ☐
Location(s) F ☐ C ☐ P ☐ T ☐ O ☐ R ☐ L ☐
no: 0-10 ☐ 10-50 ☐ >50 ☐
Runs off 5-10" ☐ Runs>10" ☐

Parox. Act., other
(Describe)
_____
_____
_____
_____
_____
_____

H.V.
No sig. Δ ☐
↑Previous ABN. ☐
Evokes New ABN. ☐
(Describe)
_____
_____
_____
_____

P.S.
No Activation ☐
Activation ☐
(Describe)
_____
_____
_____
_____

Sleep
No sig. Δ ☐
↑ Previous ABN. ☐
↓ Previous ABN. ☐
New ABN. ☐
(Describe)
_____
_____
_____
_____
_____
_____

EEG Diagnosis
Normal ☐
Abnormal ☐
Mild. ☐
Mod. ☐
Severe ☐

Type of Abnormality
Slow Waves ☐
Paroxysmal Activity ☐
Mainly φ ☐
Mainly δ ☐
Focal ☐ Generalized ☐
Location _____

Technical Quality
Acceptable ☐
Excess Artefact ☐

Comments
Improved since Previous EEG ☐
Deterioration since Previous EEG ☐
No chance since Previous EEG ☐
_____
_____
_____
_____

The rating system (Table V) described slowing and other background abnormalities and the various types of epileptiform activity (sharp waves, spikes and spike-wave complexes) and allowed these to be categorized as generalized or focal and quantified on a 3-period scale.

Table VI summarizes those clinical findings that showed significant differences or at least interesting trends between the relapse and seizure-free groups. Most of these were time-related and to a large extent correlated with each other.

TABLE VI

Summary of clinical variables apparently related to outcome

	RELAPSE	SEIZURE-FREE	P*
Age at withdrawal (mean yr)	35	28	< 0.02
Age at onsets (mean yr)	12	9	< 0.1
Age at last sz. (mean yr)	29	20	< 0.005
Duration of epilepsy (mean yr)	17	12	< 0.01
2ry generalized epilepsy	14%	0%	< 0.1
Low IQ (70-80)	22%	9%	
One seizure type only	28%	43%	
Clustering of seizures	42%	22%	

* Mann-Whitney test

TABLE VII

Summary of pharmacological findings

	RELAPSE	SEIZURES-FREE	P*
Monotherapy	29%	48%	
Carbamazepine	46%	52%	
Dose (mean mg)	626	500	
Level (mean µg/ml)	6.1	5.4	
Phenytoin	37%	43%	
Dose (mean mg)	199	108	
Level (mean µg/ml)	4.9	3.3	
Valproate	51%	33%	
Dose (mean mg)	1100	770	< 0.1
Level (mean µg/ml)	54	33	
Phenobarbitone	37%	38%	
Dose (mean mg)	160	58	< 0.1
Level (mean µg/ml)	23	4.6	< 0.1
Primidone	20%	0%	< 0.03
Dose (mean mg)	400	-	
Level (mean µg/ml)	4.7	-	
"Level"	6.4	3.7	< 0.0002

* Mann-Whitney test

The findings relating to antiepileptic drugs in the two outcome groups are summarized in Table VII. All patients taking primidone relapsed and the use of phenobarbitone was also associated with an adverse outcome. As total AED use might be more relevant than any individual drug, we divised the composite variable "Level", scoring each individual drug as 1-2 subtherapeutic, 3-4 therapeutic or 5 toxic and totalling the results. This indeed showed a highly significant effect, the mean value being substantially greater in patients who relapsed.

TABLE VIII

Summary of initial EEG findings

	Relapse	Seizure-free	P*
POST CENTRAL DOMINANT FREQUENCY (MEAN HZ)	9	9	
EXCESS THETA ACTIVITY			
Diffuse	63%	86%	
Focal	27%	14%	
DELTA ACTIVITY			
Diffuse	2%	10%	
Focal	15%	5%	
SHARP WAVES			
Diffuse	2%	19%	< 0.05
Focal	59%	71%	
Total	59%	81%	< 0.1
SPIKES			
Focal	20%	14%	
SPIKE-WAVE COMPLEXES			
Diffuse	12%	10%	
Focal	10%	0%	
ALL EPILEPTIFORM ACTIVITY			
Diffuse	63%	81%	
Focal	63%	71%	
Total	63%	81%	
SPIKES OR SPIKE-WAVE ACTIVITY			
Diffuse	29%	19%	
Focal	22%	14%	
Total	29%	19%	

*Mann-Whitney test

The EEG findings summarized here hardly support the view that the EEG is predictive of outcome. Epileptiform activity as a whole was more common in those who remained seizure-free. This effect was due to an excess of sharp waves in the seizure-free group but if these are excluded from the definition of epileptiform activity, there remains only a very weak and non-significant association of epileptiform discharges with relapse. Serial EEG stu-

dies showed a significant increase in post-central dominant frequency in both groups but no significant differences between the seizure-free and the relapse groups and in particular no tendency towards EEG deterioration in the latter.

Various associations found between clinical or laboratory variables and outcome were all too weak to be of predictive value and a multivariate analysis of the data was therefore attempted using stepwise logistic regression analysis.

TABLE IX

Summary of actual outcome, and of possible outcome if the model had been used to determine whether or not to attempt AED withdrawal

	Withdrawal in all patients	Withdrawal if score less than 0.7
Require AEDs to remain seizure-free		
Relapse	41	14
Continue AEDs	0	27
Do not require AEDs		
AEDs withdrawn	21	21
AEDs continued	0	0
Withdrawal attempted	62	34
Inappropriate treatment		
Relapsed	41 (66%)	14 (23%)
Taking AEDs unnessarily	0	0
Total	41 (66%)	14 (23%)

This did indeed produce a model with which it was possible to identify in the population of this study all patients who remained seizure-free, at cost of failing to identify 14 of the 41 who relapsed. This statistical model made use of the following variables: the number of different AEDs being taken (DRUGNUM), the cumulated serumlevels (LEVEL), and the age at the last seizure (AGELS). You will note that the variable EEG made no contribution to this model. Tabel IX summarizes the implications of adopting a strategy based on the use of such a statistical model, in fact we undertook AED withdrawal in all patients and 66% relapsed. Had we not undertaken AED withdrawal in those patients in whom the model predicted relapse, then AED withdrawal would have been attempted in only 34 patients out of the 62 but only 14 or 23% of the entire population would have relapsed. For

TABLE X

The probability of relapse with 95% confidence limits, for various combinations of values of DRUGNUM, LEVEL and AGELS

			Probability of relapse (%)	95% confidence limits
DRUGNUM 1	LEVEL 2	AGELS 10	13	3-40
		20	33	14-59
		30	63	32-86
	4	10	48	22-74
		20	76	51-90
		30	91	69-98
2	2	10	2	0-21
		20	7	3-33
		30	22	6-56
	4	10	13	3-42
		20	34	17-57
		30	64	43-81
	6	10	49	21-76
		20	76	55-90
		30	92	75-98
3	4	10	3	0-28
		20	8	1-42
		30	23	5-63
	6	10	14	2-56
		20	35	11-71
		30	65	34-87
	8	10	50	13-87
		20	77	42-94
		30	92	69-98

any given combination of the number of antiepileptic drugs (DRUGNUM), the cumulated serum level (LEVEL) and the age at last seizure (AGELS) the probability of relapse with 95% confidence limits, is given in Table X. It will be noted, not only that the findings can, in the first instance, only be regarded as applicable to the particular type of patients studied, but also that the model might well be invalid in a practice where AEDs were always given in dosages producing "therapeutic blood levels" regardless of clinical response at lower dosage. The model developed in this study needs to be tested prospectively in a larger population. An opportunity may be provided by a multi centre trial of AED withdrawal in at least 1000 seizure-free patients, recently begun in the United Kingdom.

Reference

Overweg, J. Withdrawal of antiepileptic drugs in seizure-free adult patients. Prediction of outcome. Thesis, 1985.

A POSSIBLE ROLE OF HIPPOCAMPAL PHOSPHOPROTEINS IN EPILEPTOGENESIS

L.H. Schrama, P.N.E. de Graan, F.H. Lopes da Silva, W.J. Wadman and W.H. Gispen

Introduction

Synapses in many parts of the brain show a certain degree of plasticity. Plasticity is the ability of a synaptic system to adapt its response to electrical stimuli as a function of previous experiences. Synaptic plasticity can be observed when a set of fibers is electrically stimulated by a series of brief pulses at relatively high frequencies, i.e. a tetanus. After such a tetanus, the corresponding synapses may present an enhancement in their responses to test stimuli for a period of time that can last from hours to even days. This phenomenon is called long-term potentiation (LTP). It may be considered as the physiological substrate for information storage in the brain (Bliss 1979; Bliss and Dolphin 1982; Lopes da Silva et al. 1982a,b; Eccles 1983; Voronin 1983; Teyler and Discenna 1984; Lynch and Baudry 1984).

A new dimension was added to the LTP research after the discovery that daily application of a tetanus to various regions of the brain may cause epileptiform activity and will eventually lead to generalized convulsions (Goddard et al. 1969). This experimental model is known as the kindling model of epilepsy.

Several lines of evidence point to a crucial role of calcium in epileptogenesis and the related phenomenon LTP. It has been proposed (Meldrum 1981, 1983) that an excessive influx of Ca^{2+} into selectively vulnarable cells, associated with paroxysmal activity during status epilepticus, overwhelms the capacity of the neuron to extrude or sequester Ca^{2+}, leading to an excessively high cytosolic Ca^{2+} concentration. Indeed convulsive drugs induce calcium accumulation in hippocampal neurons visualized by the oxalate-pyroantimonate technique (Griffiths et al. 1982). Moreover, we have demonstrated an increased CA^{2+}-permeability of hippocampal pyramidal cells after kindling using ion-selective micropipets (Wadman et al. 1985). An increased Ca^{2+}-influx has also been demonstrated concomitant with LTP (Baimbridge and Miller, 1981), and is thought to result in the activation of CA^{2+}-dependent proteases and the unmasking of glutamate receptors (Lynch and Baudry 1984).

The molecular mechanism(s) underlying epileptogenesis at the level of the synapse and the synaptic membrane are still largely unknown. Our research is directed toward the role of protein

phosphorylation in epileptogenesis and LTP. A general concept has developed from many observations that cyclic phosphorylation and dephosphorylation of proteins plays an important role in the regulation of ion permeability and synaptic transmission (for a review see Nestler and Greengard 1984). The early studies by Heald (1957, 1962) and Trevor and Rodnight (1965) demonstrated that in association with spike activity, serine residues of neuronal membrane proteins were phosphorylated (see also Reddington and Rodnight 1972). Since then, it has been shown that a variety of extracellular signals produce many of their diverse physiological responses by regulating the state of phosphorylation of especially membrane- or vesicle-bound substrate proteins (Oestreichter et al. 1982, Nestler and Greengard 1984).

However, the precise relation between neurotransmission and phosphorylation is not yet clear. One of the hypotheses is that the increase in intracellular CA^{2+} concentration, which is essential for neurotransmission, mediates Ca^{2+}-sensitive protein phosphorylation. Indeed, evidence from DeLorenzo and coworkers (DeLorenzo et al. 1982) suggests that the increase in intracellular Ca^{2+} stimulates the phosphorylation of cytoskeletal elements, possibly modifying cytoskeletal function and facilitating neurotransmission. Moreover, Wasterlain and Farber (1982, 1983) have shown that septal kindling results in dramatic changes in the Ca^{2+}/calmodulin sensitive phosphorylation of a 50 kDa protein.

We have studied two models for epileptogenesis in the transverse hippocampal slice system. This in vitro system has been widely used for the analysis of possible correlative changes in its electrophysiological and neurochemical properties. The hippocampal slice system combines well defined electrophysiological parameters (it contains an intact trisynaptic pathway) with a good accessability for biochemical and pharmalogical techniques. In this in vitro hippocampal slice system we induced (semi)permanent changes in electrophysiology (by applying a tetanus and the convulsant 4-aminopyridine, respectively) and studied concomitant correlative changes in protein phosphorylation.

Ltp, and the phosphorylation of a putative 52 kDa coated vesicle protein

Tetanic stimulation in the perforant path of rat hippocampal slices increased the degree of phosphorylation of a protein band with an apparent molecular weight of about 50 kDa (Bär et al. 1980). The increase in $[^{32}P]$-incorporation in the tetanized slices was 24% as compared to non-tetanized controls. Changes in other phosphoprotein bands were less consistent and reached

significance only in the 48 kDa protein, identified as the B-50 protein (Zwiers et al. 1980; see below). In further experiments it could be demonstrated that the increase in 50 kDa phosphorylation was dependent on the frequency of the tetanus. Application of 1 pulse per 4 s. for 15 min (225 pulses), a frequency which is ineffective in producing LTP, instead of 15 pulses per s. for 15 s., did not induce significant changes in 50 kDa or 48 kDa phosphorylation (Bär et al. 1980). In the absence of extracellular Ca^{2+}, a condition which inhibits neurotransmission and LTP production (Dunwiddie and Lynch 1979), no changes could be detected in either 50 kDa or 48 kDa phosphorylation, indicating that the observed changes relate to synaptic activity.

In a subsequent study (Bär et al. 1982) we investigated the characteristics of the 50 kDa band in more detail. The calculated apparent molecular weight using a high resolution separation system was 52 kDa. In fact the so-called 50 kDa band separated into a doublet with estimated molecular weights of 52 kDa and 50 kDa, respectively. Only the 52 kDa band was shown to be affected by tetanic stimulation (Bär et al. 1982).

Since the electrophysiological changes of the evoked response to single test stimuli after a tetanus may vary considerably in amplitude, we attempted to make a quantitative correlation between the changes in amplitude of the post-synaptic potential (PSP) and the population spike (PopSP) measured extracellularly, and the degree of phosphorylation of the 52 kDa band (Tielen et al. 1983). In this study each slice was tested for clear responses to test stimuli and for each slice a stimulus-responses relationship of the PSP was made, as well as a determination of the PopSP threshold. Two high frequency stimulations (50 pulses/s. for 2 s.) were given 5 min apart and 10 min after the last tetanus each slice was processed separately and assayed for endogenous protein phosphorylation. Paired control slices from the same hippocampus received test stimuli but no tetanus. The mean post hoc endogenous phosphorylation of the 52 kDa protein band was significantly increased in the tetanized group as compared to the control (+ 24%; $P < 0.05$). These data confirmed our earlier study (Bär, et al. 1980). In Fig. 1 the percentual change in 52 kDa phosphorylation is related to the change in postsynaptic potential per individual slice. A semi-logarithmic plot of these data fits a straight line with a correlation coefficient of 0.71 ($P < 0.005$). These data suggest that there may be a quantitative correlation between electrophysiological synaptic changes and synaptic membrane protein phosphorylation. However, the causality of this relationship remains to be determined.

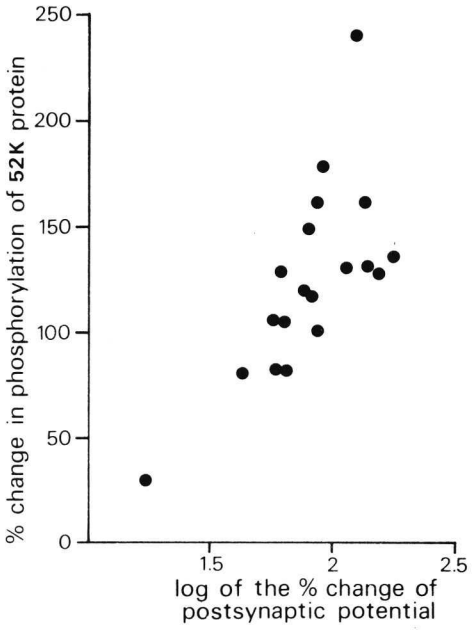

Fig. 1. Relationship between the postsynaptic potential and the endogenous phosphorylation of the 52 kDa protein, 15 min after tetanization of the perforant path fibers. Protein phosphorylation was assayed post hoc using [γ-^{32}P]-ATP in a crude synaptosomal/mitochondrial fraction (Tielen et al. 1983).

To further characterize the 52 kDa protein band we studied its subcellular localization (Bär et al. 1982). First of all we could show that the 52 kDa protein band is present in a crude synaptosomal plasma membrane fraction (t-SPM) prepared from tetanized slices and that the phosphorylation of the 52 kDa band in this t-SPM is stimulated as compared to controls (+ 30%; $P < 0.02$). A more purified synaptosomal plasma membrane fraction (as judged by electron microscopy), the so-called light synaptosomal plasma membrane fraction (l-SPM), was also found to be rich in 52 kDa phosphorylation (Fig. 2, lanes 1). Two-dimensional separation of l-SPM proteins revealed that the 52 kDa protein has an IEP of the IEF gel, thus producing a streak in the basic region of the gel. This phenomenon appears to be due to solubilization conditions (Schrama et al. in preparation) and is subject of further study.

The phosphorylation of the 52 kDa protein is not dependent on CA^{2+}/calmodulin. The [^{32}P]-incorporation into the 52 kDa protein in a crude mitochondrial/synaptosomal fraction is not affected when the Ca^{2+}-concentration during the phosphorylation assay is varied from 0-50 mM exogenous Ca^{2+} in the presence of 1 mM EGTA (Bär et al. 1982), conditions in which the 50 kDa protein and B-50 (48 kDa) show marked Ca^{2+} dependency. The in vitro endogenous phosphorylation of the 52 kDa protein is not affected by

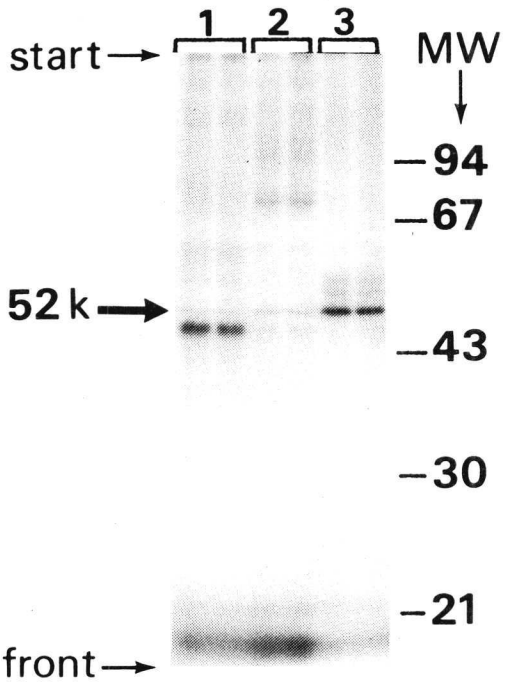

Fig. 2. Autoradiogram showing the phosphorylated 52 kDa protein in 1-SPM (1), a vesicle-enriched fraction (2) and isolated coated vesicles (3) after protein separation on 11% SDS-polyacrylamide gels. The position of molecular weight standards (MW) is indicated at the right.

cAMP either. Similarly, we have found the 52 kDa phosphorylation in 1-SPM to be Ca^{2+}- and cAMP-independent (De Graan unpublished observations).

Recently, the phosphorylation of a protein in the 50 kDa range in purified bovine brain coated vesicles has been described (Pauloin et al. 1982; Kadota et al. 1982; Pauloin and Jolles 1984). Our attention was drawn to this 50 kDa protein called pp50 since it shares with our 52 kDa protein its insensitivity to Ca^{2+}/calmodulin and cAMP (Pauloin et al. 1982; Bär et al. 1982). Subcellular localization studies show that 52 kDa phosphorylation can be detected in the 1-SPM fraction from rat brain (Bär et al. 1982), and is very pronounced in a vesicle-enriched fraction prepared from synaptosomes (Fig. 2, lanes 2). Therefore, we isolated coated vesicles from rat brain according to the method of Pearse and Robinson (1984). The major phosphoprotein in this coated vesicle preparation was found to be a 52 kDA protein (when analyzed on 11% SDS-polyacrylamide gel),. which comigrates with our 52 kDa protein from 1-SPM (Fig. 2, compare lanes 1 to lanes 3). Moreover, the phosphorylation of the 52 kDa coated vesicle pro-

tein was not affected by Ca^{2+} and calmodulin or cAMP. Preliminary data from two-dimensional separation systems and peptide mapping indicated that both 52 kDa proteins are related if not identical.

Coated vesicles are regarded as highly specialized subcellular organelles, which are involved in receptor-mediated endocytosis (for review see Goldstein et al. 1979). Receptor-mediated endocytosis is an important and general mechanism by which animal cells take up nutrients and regulatory proteins. Proteins which bind to plasma membrane receptors are rapidly internalized, by clustering of the receptors in specialized regions of the plasma membrane, called coated pits, that invaginate rapidly into the cell during endocytosis to form coated vesicles. Most data on coated pits and coated vesicles are derived from studies on the low density lipoprotein receptor system (Goldstein et al. 1979). In brain the synaptic vesicle membrane has been postulated to be selectively retrieved from the presynaptic membrane by receptor-mediated endocytosis (Heuser and Reese 1973). More recent data indicate indeed that coated pits and coated vesicles are involved in presynaptic membrane recycling (Heuser and Reese 1979; Kadota and Kadota 1982). Although the precise role of pp50 phosphorylation still remains unclear, its presence in various different coated vesicle species (Pauloin et al. 1984) suggests an important role in the coated-vesicle working mechanism, like regulating selective internalization, repeated membrane fusion and fission. As the 52 kDa protein in 1-SPM is related or identical to the pp50 protein, it may well be that the mechanism of LTP is linked to presynaptic coated vesicle function. Hence modulation of 52 kDa phosphorylation in response to tetanic stimulation may thus be related to an increase in presynaptic membrane renewal and an increased neurotransmisser turnover (see Fig. 5).

4-aminopyridine and modulation of Ca^{2+}/calmodulin-dependent protein phosphorylation

Several lines of evidence point to a crucial role of calcium in epileptogenesis and in general convulsant drugs, such as 4-aminopyridine, induce calcium accumulation in hippocampal neurons (Griffiths et al. 1982). The precise mechanism by which aminopyridines increase intracellular calcium is not yet identified, although it is now clear that the mechanism is distinct from that of the dihydropyridines, a group of calcium entry activators, e.g. Bay K 8644 (Greenberg et al. 1984). The convulsant 4-aminopyridine (4-AP) is thought to induce epileptoform activity by blocking the potassium channels associated with an influx of calcium (Llinas et al. 1976; Nicholson et al. 1976; Baranyi and Feher 1979; Pant et al. 1983). This influx of calcium results in an increase in synaptic transmitter release in the peripheral and

central nervous system, leading to prolongation of action potentials in unmyelinated nerve fibers and terminals (Thesleff 1980; Haas et al. 1983). The effects of 4-aminopyridine have been shown in both excitatory and inhibitory neurotransmission (Jankowska et al, 1977; Buckle and Haas, 1982; Van Harreveld 1984). These effects of the convulsant have been confirmed by morphological changes in 4-aminopyridine treated synapses (Tokunaga et al. 1979; Forsman and Elfvin 1983).

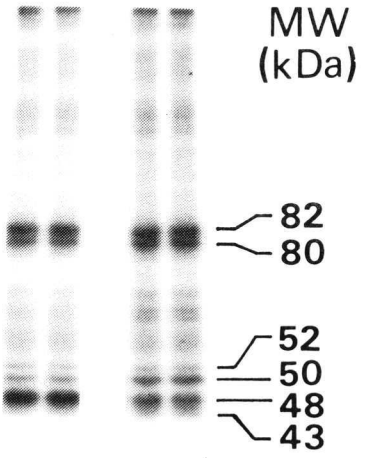

Fig. 3. Autoradiogram of a phosphorylated crude mitochondrial/synaptosomal fraction after incubation of hippocampal slices for 30 min in the absence (A) or presence (B) of 10^{-5} M 4-AP. Indicated are the estimated molecular weights of the six major phosphoproteins.

A B

After treatment of hippocampal slices with 10^{-5} M 4-AP, a pronounced increase was found in the phosphorylation of the 50 kDa protein (Fig. 2). Quantification of the [^{32}P] incorporation after incubation with 10^{-5} M 4-AP (Fig. 3) revealed a stimulation of 86% of the 50 kDa phosphorylation, whereas a smaller but significant stimulation was found of the 80 kDa phosphorylation. The 48 kDa protein was inhibited by 4-AP with a maximal effect at 10^{-4} M of 20%. The 4-AP induced stimulation of 50 kDa phosphorylation was dose-dependent (Fig. 4), with a half maximal stimulation of 5×10^{-7} M. The effect of the convulsant on 80 kDa phosphorylation was only significant at 10^{-5}M. No effect could be detected

on the phosphorylation of other major phosphoproteins (82, 52 and 43 kDa) at any of the 4-AP concentrations tested.

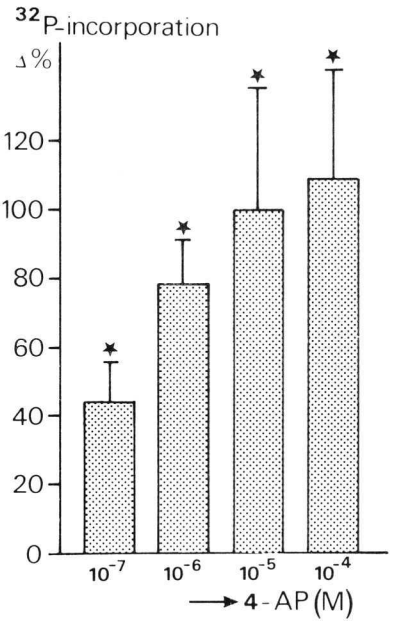

Fig. 4. Quantification of the effect of 10^{-5} M 4-AP as shown on the autoradiogram in Fig. 1 (n = 13). Quantification was performed by densitometric scanning of the autoradiogram and measured as peak height above background. The data are expressed as percentage of controls. Bars indicate SEM values. * = $2p = < 0.05$ as determined with Students t-test.

The conditions leading to enhancement of 50 kDa protein phosphorylation in the post hoc phosphorylation assay resemble those leading to spontaneous epileptic activity by 4-AP in hippocampal slices both with respect to dose- and time-dependency of the effect (Buckle and Haas 1982; Haas et al. 1983; Van Harreveld 1984).

The 50 kDa phosphoprotein band described here is the same as the one described earlier by Bär et al. (1982). The phosphorylation of this protein is not affected by cyclic AMP or tetanic stimulation, in sharp contrast to the 52 kDa protein also present in the crude mitochondrial/synaptosomal fraction (Bär et al. 1980; Lopes da Silva et al. 1982a,b; Tielen et al. 1983). The phosphorylation of this 50 kDa protein is, however, strongly dependent on the presence of calcium ions and the calcium binding protein calmodulin (Bär et al. 1982).

In another experimental model for epilepsy, the kindling model, a marked increase in ^{32}P incorporation into a 50 kDa hippocampal protein is reported (Wasterlain and Farber 1982). The phosphory-

lation of this protein and the effect of kindling are calmodulin-dependent (Wasterlain and Farber 1984). Biochemical characterization showed that the protein is probably the alpha or rho subunit of the calcium/calmodulin-dependent protein kinase II (Goldenring et al. in press). This protein kinase is a premoninant brain phosphoprotein (Bennett et al. 1983), consisting of two subunits, with a characteristic distribution between the subunits over the brain (McGuinness et al. 1985). The major post-synaptic density protein has been identified as the alpha or rho subunit of brain calcium/calmodulin-dependent porotein kinase (Kennedy et al. 1983). Moreover, this protein kinase is associated with cytoskeletal components of post-synaptosomal fractions (Sahyoun et al. 1985).

The 50 kDa phosphoprotein which is affected by 4-AP treatment of hippocampal slices is most probably the autophosphorylated alpha or rho subunit of the brain calcium/calmodulin-dependent protein kinase, on basis of its molecular weight range and its calcium/calmodulin sensitivity (Bär et al. 1982). The precise mechanism of 4-AP action is not yet clear, but might involve the influx of calcium leading to a change in the autophosphorylation of the α-subunit of the calcium/calmodulin-dependent protein kinase. Autophosphorylation of the kinase probably modulates the activity of the enzyme, thus affecting cytosekeletal structure and synaptic transmission.

Concluding remarks

In recent years, several different mechanisms have been proposed to underly epiletogenesis. These include 1) an increase in the Ca^{2+}-permeability of the neruonal membrane, resulting in an elevation of cytosolic Ca^{2+} (Meldrum 1981, 1983; Griffith et al. 1982, Wadman et al. 1985), 2) an increase in extracellular K^{+}, possibly resulting from a reduced spatial buffering (Heineman et al. 1977, 1983), 3) a decrease in GABA-ergic inhibition (Meldrum 1983; Rondouin et al. 1983) and 4) blockage of Ca^{2+}-dependent K^{+} channels (Galvan et al. 1982; Agoston et al. 1983). We have focussed our research on a possible role of phosphorylation processes in epileptogenesis with special emphasis on Ca^{2+}-dependent phosphorylation. Both in the LTP model and in the 4-AP model we have shown that characteristic electrophysiological changes are parallelled by changes in the phosphorylation of specific synaptic proteins.

In the LTP model, primarily the phosphorylation of a 52 kDa protein was affected, which appears to be identical to the pp50 protein in coated vesicles. This protein is thought to play a role in modulating coated vesicle function, i.e. receptor-mediated

endocytosis and membrane renewal (Fig. 5). In the 4-AP model, phosphorylation was confined to a 50 kDa protein, tentatively identified as the α-subunit of the Ca^{2+}/calmodulin-dependent protein kinase type II. The autophosphorylation of this kinase is thought to regulate kinase activity. The kinase is involved in the modulation of cytoskeletal function and synaptic transmission (Fig. 5).

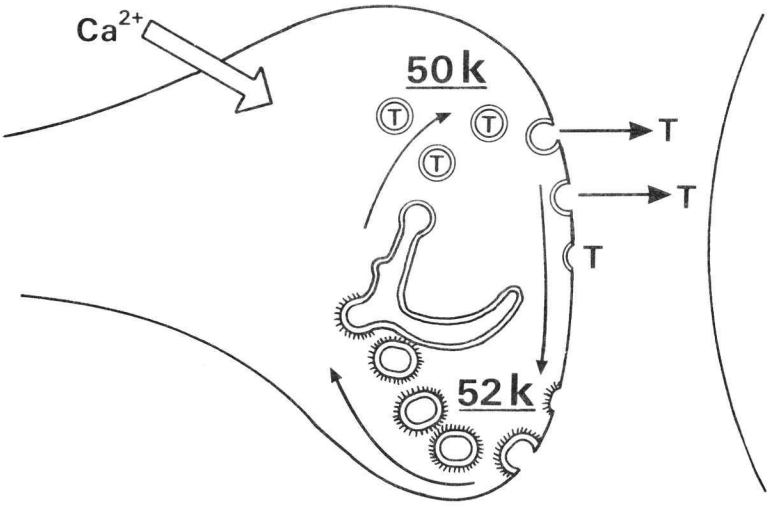

Fig. 5. Schematic representation of the putative role of the 52 kDa and 50 kDa protein in epileptogenesis. The 52 kDa protein is found in the synaptic plasma membrane and the coated vesicles. This protein is thought to play a modulatory role in receptor-mediated endocytosis and membrane renewal. The 50 kDa protein appears to be the α-subunit of Ca^{2+}/calmodulin-dependent protein kinase type II. This kinase may play a regulatory role in cytoskeletal function and possible in neurotransmission.

(T) = neurotransmitter

In both the LTP and 4-AP model the phosphorylation of the nervous tissue specific, presynaptic protein B-50 (for review see Gispen et al. 1985) is affected. In the LTP model we initially described a rather variable increase in B-50 phosphorylation (Bär et al. 1982), but more recently Routtenberg and coworkers (Lovinger et al. 1985; Routtenberg and Lovinger 1985) have reported a selective increase in the phosphorylation of protein F_1 (which we believe to be identical to B-50) in an in vivo LTP model. Indeed, we have recently been able to demonstrate a good correlation in the hippocampal slice LTP model, between the degree of potentiation and the increase in B-50 phosphorylation (unpublished results). In the 4-AP model a consistant decrease in B-50 phosphorylation has been observed (see above). B-50 is thought to

play a role in the modulation of receptor-mediated breakdown of polyphosphoinositides (for a review see Gispen et al. 1985) and thus in the mobilization of Ca^{2+} as a second messenger.

Thus, we have reported changes in the degree of phosphorylation of 3 different neuronal proteins, which parallel changes seen in electrophysiological parameters of the neuronal network involved. We realize that correlative studies only provide indirect evidence for a role of protein phosphorylation in epileptogenesis. A causal relationship has yet to be established. The in vitro experimental models for epilepsy used in our studies are very suitable to investigate the effects of anti-epileptic drugs on protein phosphorylation. By doing so, we hope to contribute to the understanding of the molecular mechanisms underlying epileptogenesis and the mechanism of action of antiepileptic drugs.

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References

Agoston, D., Hargittai, P. and Nagy, A. Effects of 4-amino pyridine in calcium movements and changes in membrane potential in pinched-off nerve terminals from rat cerebral cortex. J. Neurochem., 1983, 41: 745-751.

Baimbridge, K.G. and Miller, J.J. Calcium uptake and retention during long-term potentiation of neuronal activity in the rat hippocampal slice preparation. Brain. Res., 1981, 221: 299-305.

Bär, P.R., Schotman, P., Gispen, W.H., Lopes da Silva, F.H. and Tielen, A.M. Changes in synaptic membrane phosphorylation after tetanic stimulation in the dentate area of the rat hippocampal slice. Brain Res. 1980, 198: 478-484.

Bär, P.R., Tielen, A.M., Lopes da Silva, F.H., Zwiers, H. and Gispen, W.H. Membrane phosphoproteins of rat hippocampus: Sensitivity to tetanic stimulation and enkephalin. Brain Res. 1982, 245: 69-79.

Baranyi, A. and Feher, O. Convulsive effects of 3-aminopyridine on cortical neurons. Electroencephal. Clin. Neurophysiol., 1979, 47: 745-751.

Bennett, M.K., Erondu, N.E. and Kennedy, M.B. Purification and characterization of a calmodulin-dependent protein kinase that is highly concentrated in brain. J. Biol. Chem., 1983, 258: 12735-12744.

Bliss, T.V.P. Synaptic plasticity in the hippocampus. Trends in Neurosc. 1979, 2: 42-45.

Bliss, T.V.P. and Dolphin, A.C. What is the mechanism of long-term potentiation in the hippocampus? Trends in Neurosc., 1982, 5: 289-290.

Buckle, P.J. and Haas, H.L. Enhancement of synaptic transmission by 4-aminopyridine in hippocampal slices of the rat. J. Physiol., 1982, 326: 109-122.

DeLorenzo, R.J., Gonzalez, B., Goldenring, J., Bowling, A. and Jacobson, R. Ca^{2+}-calmodulin tubulin kinase system and its role in mediating the Ca^{2+} signal in brain. Progr. Brain Res., 1982, 56: 255-286.

Dunwiddie, T.V. and Lynch, G. The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. Brain Res., 1979, 169: 103-110.

Eccles, J.C. Calcium in long-term potentiation as a model for memory. Neuroscience 1983, 10: 1071-1081.

Forsman, V.C. and Elfvin, L.-G. An ultrastructural study of presynaptic membrane specializations in sympathetic ganglia of 4-aminopyridine treated guinea pigs and rats. Brain Res., 1983, 208: 355-360.

Galvan, M., Grafe, P. and Ten Bruggencate, G. Convulsant actions of 4-aminopyridine on the guinea pig olfactory cortex slice. Brain Res., 1982, 241: 75-86.

Gispen, W.H., Van Dongen, C.J., De Graan, P.N.E., Oestreicher, A.B. and Zwiers, H. The role of phosphoprotein B-50 in phosphoinositide metabolism in brain synaptic plasma membranes. In: Bleasdale, J.E., Eichberg, J. and Hauser, G. (eds.). Inositol and Phosphoinositides, Metabolism and Regulation, Humana Press, New Yersey pp. 399-414 (1985)

Goddard, G.V., McIntyre, P.C., Leech, C.K. A permanent changeing brain function resulting from daily electrical stimulation. Exp. Neurol., 1969, 25: 295-330.

Goldenring, J.R., Wasterlain, C.G., Oestreicher, A.B., De Graan, P.N.E., Farber, D.B., Glaser, G. and DeLorenzo, R.J. Kindling induces a long lasting change in the activity of a hippocampal membrane calmodulin-dependent protein kinase system. Brain Res., in press.

Goldstein, J.L., Anderson, R.G.W. and Brown, M.S. Coated pits, coated vesicles, and receptor-mediated endocytosis. Nature (London), 1979, 279: 679-685.

Greenberg, D.A., Cooper, E.C. and Carpenter, C.L. Calcium entry activators: distinct sites of dihydropyridine and aminopyridine action. Neuroscience Lett., 1984, 50: 279-282.

Griffiths, T., Evans, M.C. and Meldrum, B.S. Intracellular sites of early calcium accumulation in the rat hippocampus during status epilepticus. Neurosc. Lett., 1982, 30: 329-344.

Haas, H.L., Wieser, H.G. and Yasargil, M.G. 4-Aminopyridine and fiber potentials in rat and human hippocampal slices. Experientia, 1983, 39: 114-115.

Heinemann, U., Lux, H.D. and Gutnick. Extracellular free calcium and potassium during paroxysmal activity in the cerebral cortex of the cat. Exp. Brain Res., 1977, 27: 237-243.

Heinemann, U., Neuhaus, S., Dietzel, I. Aspects of potassium regulation in normal and epileptic brain tissue. In: Baldy-Mouliner, M., Ingvar, D.A., Meldrum, B.S. (eds.). Current problems in epilepsy. John Libbey, London, Paris pp. 271-277 (1983).

Heald, P.J. The incorporation of phosphate into cerebral phosphoprotein promoted by electrical impulses. Biochem. J., 1957, 66: 659-663.

Heald, P.J. Phosphoprotein metabolism and ion transport in nervous tissue: A suggested connexion. Nature (London), 1962, 193: 451-454.

Heuser, J.E. and Reese, T.S. Evidence for recycling of synaptic vesicle membranes during transmitter release at the frog neuromuscular junction. J. Cell Biol, 1973, 47: 315-344.

Heuser, J.E. and Reese, T.S. Synaptic vesicle exocytosis captured by quick freezing. In F.O. Schmitt and F.G. Worden (eds.), The Neurosciences Fourth Study Program, Cambridge: Massachusetts Institute of Technology Press, pp. 572-600 (1979).

Jankowska, E., Lundberg, A., Rumodin, P. and Sykova, E. Effects of 4-aminopyridine on transmission in excitatory and inhibitory synapses in the spinal cord. Brain Res., 1977, 136: 387-392.

Kadota, T. and Kadota, K. Membrane retrieval by macropinocytosis in presynaptic terminals during transmitter release in cat sympathetic ganglia in situ. J.Elect.Microsc., 1982, 31: 73-80.

Kadota, K., Usami, M. and Takahashi, A. A protein kinase and its substrate associated with the outer coat and the inner core of coated vesicles from bovine brain. Biomed. Res., 1982, 3: 575-578.

Kennedy, M.B., Bennett, M.K. and Erondu, N.E. Biochemical evidence that the "major post-synaptic density protein" is a subunit of a calmodulin-dependent protein kinase. Proc. Natl. Acad. Sci. USA. 1983, 80: 7357-7361.

Llinas, R., Walton, K. and Bohr, V. Synaptic transmission in squid giant synapse after potassium conductance blockage with external 3- and 4-aminopyridine. Biophys. J., 1976, 16: 83-86.

Lopes da Silva, F.H., Bär, P.R., Tielen, A.M. and Gispen, W.H. Plasticity in synaptic transmission and changes of membrane-bound protein phosphorylation. Progr. Brain Res., 1982a, 55: 369-377.

Lopes da Silva, F.H. Bär, P.R., Tielen, A.M. and Gispen, W.H. Changes in membrange phosphorylation correlated with long-lasting potentiation in rat hippocampal slices. Progr. Brain Res., 1982b, 56: 339-347.

Lovinger, D.M., Akers, R.F., Nelson, R.B., Barnes, C.A., McNaughton, B.L. and Routtenberg, A. A selective increase in phosphorylation of protein F1, a protein kinase C substrate, directly related to three day growth of long-term synaptic enhancement. Brain Res., 1985, 343: 137-143.

Lynch, G. and Baudry, M. The biochemistry of memory: A new and specific hypothesis. Science, 1984, 224: 1057-1063.

McGuinness, T.L., Lai, Y., Greengard, P. Ca^{2+}/calmodulin dependent kinase II. Isozymic forms from rat forebrain and cerebellum. J. Biol. Chem., 1985, 260: 1696-1704.

Meldrum, B.S. Metabolic effects of prolonged epileptic seizures and the causation of epileptic brain damage. In: F.C. Rose (ed.), Metabolic disorders of the nervous system. Pitman Medical London pp. 175-187 (1981).

Meldrum, B. Convulsant and anticonvulsant drug mechanisms. In: Baldy-Mouliner, M., Ingvar, D.A., Meldrum, B.S. (eds.). Current problems in epilepsy. John Libbey, London, Paris pp. 324-335 (1983).

Nestler, E.J. and Greengard, P. (eds.) Protein Phosphorylation in the nervous system. John Wiley Inc., New York (1984).

Nicholson, C., Steinberg, R., Stockle, H. and Ten Bruggecate, G. Calcium decrease associated with 4-aminopyridine-induced potassium increase in cat cerebellum. Neuroscience Lett., 1976, 3: 315-319.

Oestreicher, A.B., Zwiers, H. and Gispen, W.H. Synaptic membrane phosphorylation: Target for neurotransmitters and peptides. Progr. Brain Res., 1982, 55: 349-367.

Pant, H.C., Gallant, P.E., Cohen, R., Neary, J.T. and Gainer, H. Calcium-dependent 4-aminopyridine stimulation of protein phosphorylation in squid optic lobe synaptosomes. Cell. Molec. Neurobiol., 1983, 3: 223-238.

Pauloin, A., Bernier, I. and Jolles, P. Presence of cyclic nucleotide Ca^{2+}-independent protein kinase in bovine brain coated vesicles. Nature (London), 1982, 298: 574-576.

Pauloin, A. and Jolles, P. Internal control of the coated vesicle pp50-specific kinase complex. Nature (London), 1984, 311: 265-267.

Pauloin, A., Loeb, J. and Jolles, P. Protein kinase(s) in bovine brain coated vesicles. Biochim. Biophys. Acta, 1984, 799: 238-245.

Pearse, B.M.F. and Robinson, M.S. Purification and properties of 100 kD proteins from coated vesicles and their reconstruction with clathrin. EMBO J., 1984, 3: 1951-1957.

Reddington, A. and Rodnight, R. Effect of putative transmitters and other agents on phosphoprotein turnover in respiring slices of guinea-pig cerebral cortex. Biochem. J., 1972, 126: 14-15.

Rondouin, G., Lerner-Natoli, M., Privat, A., Bali, J.P., Chambon J.P., Chicheporticke, R. and Baldy-Moulinier, M. GABA-ergic neurotransmission in the kindling model of epilepsy. In: Baldy-Mouliner, A., Ingvar, D.A. and Meldrum, B.S. (eds.), Current problems in Epilepsy, John Libbey, London, Paris, pp. 357-364 (1983).

Routenberg, A. and Lovinger, D.M. Selective increase in phosphorylation of a 47 kDa protein (F1) directly related to long-term potentiation. Behav. and Neural Biol., 1985, 43: 3-11.

Sahyoun, N. LeVine, III, H., Bronson, D., Siegel-Greenstein, F. and Cuatrecasas, P. Cytoskeletal calmodulin-dependent protein kinase. Characterization, solubilization, and purification from rat brain. J. Biol. Chem., 1985, 260: 1230-1237.

Teyler, T.J. and Discenna, P. Long-term potentiation as a candidate mnemonic device. Brain Res. Rev., 1984, 7: 15-28.
Thesleff, S. Aminopyridines and synaptic transmission. Neuroscience, 1980, 5: 1413-1419.
Tielen, A.M., De Graan, P.N.E., Mollevanger, W.J., Lopes da Silva, F.H. and Gispen, W.H. Quantitative relationship between posttetanic biochemical and electrophysiological changes in rat hippocampal slices. Brain Res., 1983, 277: 189-192.
Tokanuga, A., Sandri, C. and Akert, K. Ultrastructural effects of 4-aminopyridine on the presynaptic membrane in the rat spinal cord. Brain Res., 1979, 163: 1-8.
Trevor, A.J. and Rodnight, R. The subcellular localization of cerebral phosphoprotein sensitive to electrical stimulation. Biochem. J., 1965, 95: 889-896.
Van Harreveld, A. Effects of 4-aminopyridine on the field potentials of hippocampal slices. Neuroscience Lett., 1984, 50: 283-287.
Voronin, L.L. Long-term potentiation in the hippocampus. Neuroscience, 1983, 10: 1051-1069.
Wadman, W.J., Heinemann, U., Konnerth, A. and Neuhaus, S. Hippocampal slices of kindled rats reveal calcium involvement in epileptogenesis. Esp. Brain Res., 1985, 57: 404-407.
Wasterlain, C.G. and Farber, D.B. A lasting change in protein phosphorylation associated with septal kindling. Brain Res., 1982, 247: 191-194.
Wasterlain, C.G. and Farber, D.B. Kindling alters the calcium/calmodulin-dependent phosphorylation of synaptic plasma membrane proteins in hippocampus. Proc. Natl. Acad. Sci. USA, 1984, 81: 1253-1257.
Zwiers, H. Schotman, P. and Gispen, W.H. Purification and some characteristics of an ACTH-sensitive protein kinase and its substrate protein in rat brain membranes. J. Neurochem., 1980, 34: 1689-1699.

AN OVERVIEW OF 15 YEARS CLEO FUNDED RESEARCH

H. Meinardi

Otto Magnus and the author were responsible for the birth of CLEO, ably assisted by Mia Slag, general secretary of the Health Organization TNO, who fulfilled the role of midwife. At that time it was not clear whether the newborn was to grow into a goldfish or a muse. In any case a prosperous future seemed to be guaranteed.

At that time a number of questions were under discussion, i.e.:
- Is it possible to find a genetic marker which is strongly associated with primary generalized epilepsy and which is permanently present? Thus the identification of patients with primary generalized epilepsy would not be any longer dependent on the need to catch a paroxysmally occurring signal. The possible existence of such a marker had been indicated by Millichap and Ulrich (1962), who have suggested that in the urine of those suffering from primary generalized epilepsy a higher concentration of neutral amino-acids appeared to be present.
- Is it possible that the intelligence of people with epilepsy in the long run deteriorates and if so is this associated with seizures, therapy or another process?
- Can the therapeutic efficacy or lack of efficacy of anti-epileptic drugs be explained by the information obtained from the bloodlevels of these drugs in the interval between the administration of the drug and its elimination?
- Is it possible to improve the electroencephalographic technology in order to reduce false negative results?
- Is it possible to isolate "the epileptic neuron" and use it for testing of new anti-epileptic drugs, thus eliminating the multiple parameters complicating testing in the intact organism?

In the "Instituut voor Epilepsiebestrijding" there was also an interest in other questions more related to the day to day life of people with epilepsy, e.g. the question whether taking part in sports would increase or perhaps even reduce the frequency of seizures. And of course there was the continuous need to test the clinical efficacy of new anti-epileptic drugs developed by the pharmaceutical industry.

At the beginning applications for grants to CLEO were predominantly submitted from workers at the "Instituut voor Epilepsiebestrijding", or its application was stimulated by that institute. For example studies at the Sociological Institute of the University of Groningen were initiated through a first contact

between the research management of the "Instituut voor Epilepsiebestrijding and Dr. H.L. Jessen. The latter unfortunately died at a relatively young age. Post aut propter, several years went by before Mulder and Suurmeijer (CLEO A9) and Suurmeijer alone (CLEO A17) approached the epilepsy problem from a sociological point of view (CLEO A9, A 17). An important milestone was Suurmeijer's thesis "Children with Epilepsy" (1980). Presently also research from the Dr. Hans Berger Clinic was initiated with the help of CLEO. Brunia tried to obtain more insight in the neurophysiological significance of 3/s. spike-and-wave discharges through the study of the response times of the Hoffmann reflex (CLEO A 9).

From the University of Nijmegen important contributions were made with respect to the study of the turnover of anti-epileptic drugs. From this same university Hommes joined epilepsy research through the gate of the folic acid controversy (CLEO A 9, A 11, A 28, A 45, A 50). Interest in folic acid has been excited in particular by Reynolds (1983) who defends the thesis that folic acid is instrumental in the alternation between psychotic behavior and epileptic seizures. Lack of folic acid would induce psychosis while folic acid itself would favour the occurrence of seizures. Certain anti-epileptic drugs indeed reduce serum folate levels. Anti-epileptic drugs also influence levels of other important constituents, for example the level of vitamine D and calcium metabolism. This particular problem has been studied in our country by Bongers and Melman (1978).

The Institute of Medical Physics TNO under the guidance of Storm van Leeuwen became increasingly involved in the study of epilepsy. His group was particularly interested in the mechanisms of seizure spread. Lopes da Silva is still very much engaged in this matter. (CLEO A 49, this volume).
Van Parijs investigated the possibility of making use of statokinesiometry as a tool to assess possible ill effects of the chronic use of anti-epileptic drugs on stature and motorbehavior (CLEO A 13).

The immigration of Beverly Kulig to The Netherlands was a boon. Thanks to her experience in the laboratory of Calhoun she was particularly well equipped to study, with the help of animal experiments, which part is played by excessive discharge of grey matter on one hand and chronic administration of anti-epileptic drugs on the other hand as a cause for cognitive disturbances in epilepsy. In addition, it may also be possible that anti-epileptic drugs, notwithstanding their own effect, improve learning in epilepsy through suppression of seizures (CLEO A 16, A 24).
At about the same time when Kulig started her animal experimental

work, Van Zijl and subsequently Aldenkamp worked on improving the evaluation of psychological functioning in epilepsy (CLEO A 3, A 43).

Not directly sponsored by CLEO, but yet only possible thanks to the research potential in the field of epilepsy generated by CLEO, was the development of workshops in order to obtain expert opinions on different aspects of epilepsy.
These workshops that now are held under the auspices of Epilepsy International are known by such characteristic names as WODADIBOF (Meijer et al. 1972; Schneider et al. 1974; Gardner-Thorpe et al. 1976; Johannessen et al. 1979), (Workshop on Determination of Anti-epileptic Drugs), WOPSASSEPY (Kulig et al. 1980), (Workshop on Psychological Assessment of People with Epilepsy), WOMAD (Workshop on Metabolism of Anti-epileptic Drugs), (Levy et al. 1982) and so on.
During those workshops a maximum of 30 experts met to discuss a circumscribed problem of epileptology and to indicate possible fruitful approaches to reach a solution.

The WODADIBOF's which started in 1972 have made important contributions to the present practice of supporting pharmacotherapy of epilepsy with measurements of drug levels in body fluids. Grant recipients of CLEO like Meijer and Driessen have been very much involved in these meetings (CLEO A1, A 18, A 26, A 54).

With respect to anti-epileptic drugs it is not just a matter of how to use them, but it is also not clear when and how to stop using them. First Van Heycop ten Ham (CLEO A 25, A 34) took up this challenge and at present Overweg is still working at it (this volume). The present armamentarium of anti-epileptic drugs in use in The Netherlands lists about thirteen compounds of which only five are used frequently.

For many years the pharmaceutical industry has shown little interest in the production of new anti-epileptic drugs. Thanks to the efforts of the American Government this trend has changed favourably. Of the different phases of pharmacological research: - I. animal pharmacology, II. animal toxicology, III. tolerance and human pharmacokinetics in volunteers, IV. effectivity testing in thusfar therapy resistant patients - the first three fases are usually the responsibility of the industry, for the fourth the medical practitioner is responsible.

In The Netherlands clinical trials of new anti-epileptic drugs have been carried out for Valproate (Meinardi, 1971), Valpromide (not published), Cyheptamide, Mexetyline (Bongers et al. 1974), Progabide (Van der Linden et al. 1980), Oxcarbazepine (in

preparation), Milacemide (Houtkooper, in press), while studies are still in progress on Flunarizine, Lamotrigine and CGP 11952. Valproate has become one of the five major anti-epileptic drugs. However, during post-market research there has been an indication that the risk of getting a child with spina bifida increases significantly if the mother uses valproate during pregnancy. This finding, by the way, has not been part of the prospective study on children exposed to anti-epileptic drugs during pregnancy, as the cohort for this study is still too small. This latter study started in Holland in 1972 and the cohort comprises at present 323 pregnancies (Starrveld-Zimmerman 1974; Lindhout 1985). The warning for a possible association of valproate with spina bifida came from a French register for birth defects. In general, follow-up of the results of antenatal exposure to anti-epileptic drugs is limited to the period immediately after delivery. There is a great need to follow these children and their further development at least until the age of 6 years. However, at present there are not sufficient funds available to realize such a study.

The mechanism of action of most anti-epileptic drugs is still not sufficiently understood. With respect to Valproate, Mandel and co-workers (1977) first postulated a Gaba-mimetic activity due to interference with GABA catabolism. With support of CLEO Bruinvels (CLEO A 37) studied this problem and gave evidence of the important role of succinic semi-aldehyde in this process. For the understanding of epileptogenesis and suppression of seizures sooner or later the role of endogenous peptides will have to be clarified. This work has been taken up by Dzoljic who is studying the opioid peptides (CLEO A 44). This class of peptides appeared to be involved in eliciting seizures as well as in elevating seizure threshold depending on the site of application and receptor characteristics. Neuropeptide research is still in its infancy. Peptides are reported to play also a role in sleep processes.

Sleep has an important influence on paroxysmal excessive discharge of neurones. The neurophysiological analysis of sleep and epilepsy has been taken up by Declerck, first with the support of CLEO (CLEO A 32), at present with the support of the "Praeventiefonds".

Recently Van Emde Boas obtained a grant from CLEO for a study in this field with particular emphasis on the identification of EEG patterns in sleep with a diagnostic significance for epilepsy (CLEO A 53). Van Hulzen is working on an animal model to differentiate the influence of seizure perturbed sleep, respectively seizures themselves, on learning abilities (CLEO A 48).

The social aspects of epilepsy have only been scantily investigated with CLEO support. Wijnands, Wiegman and Gutteling studied attitudes towards epilepsy and the impact of different information paradigms (CLEO A 31, A 35). Smits studied the structure of health and welfare care provisions for people with epilepsy (CLEO A 21). Though not in itself a behavioural scientific approach the study of Höppener and Kuijer on the influence of social drinking (moderate alcohol consumption) on seizure frequency is of social and psychological importance (CLEO A 36).

There is a well-nigh infinite field to be worked on, limited on the one side by finding an answer to the question why neurones develop a tendency for excessive random (?) discharges and on the other side by finding an answer as to which mechanisms impede the participation of people with epilepsy in the activities of our society equal to those of people who do not have seizures or who do not have to take anti-epileptic drugs to remain seizure-free. On the basic science end of this field we find Voskuyl, Gispen, Lopes da Silva and their co-workers.
Voskuyl and Albus are looking into the changes that take place in the electrophysiological properties of neuronal membranes of hippocampal cells when they are exposed to epileptogenic substances or when the animal has been made epileptic through kindling (CLEO A 41, A 55).
Gispen and coworkers are studying the compositions of these neuronal membranes and the biochemical background of changes associated with epileptogenesis (CLEO A 42, A 47).
Lopes da Silva and his group are engaged in the analysis of seizure spread (CLEO A 33).
In the Netherlands little work is being done with respect to the role of glial cells. However, the Netherlands obviously is not the only country where scientists have an interest in epileptology. Theoretically it would be desirable that all these efforts would be coordinated world-wide. However, it is evident that already within the boundaries of a relatively small country, it is quite difficult to agree on a division of labour and a combined approach.

Recently a new complication has developed. Especially in the medical sciences it has been a tradition to make new knowledge available to everyone without restrictions. Surely sometimes some secrecy was maintained if one felt to be on one's way to epoch-making discoveries. However, in general medical knowledge was shared. At present, where the financial means to support research are dwindling, it is given serious consideration to obtain financial remuneration for the fruits of one's research. This is particularly evident for technical innovations; in the bioindustry there are already similar examples, while the pharmaceutical

industry has always worked along these lines. Personally I resent this development through which our primary goal to take care of the health of mankind may be replaced by purely commercial goals. Obviously the way back is to insure proper financial support of research which will permit research workers to concentrate on their job instead of losing time on fundraising.
I should not elaborate on this theme in this context. I have been asked by the organizer to present an overview of the achievements of CLEO, especially with respect to clinical epileptology, and not to discuss biopolitics.

I can hardly separate the progress in clinical epileptology from the total of work performed under the auspices of CLEO, to begin with grant CLEO A 1 the recipient of which, Nora Baart, studied the use of aminoacid serum levels as a marker for primary generalized epilepsy, all the way up to at present grant A 55 which was awarded for the most recent three-year plan to find out about mechanism of epilepsy with the help of isolated slices of cerebral tissue ant its reaction to epileptogenic and anti-epileptic drugs.

I have already briefly mentioned most of the projects supported by CLEO except a few which have only in part been financed by CLEO like the one of Van den Berg (CLEO A 39) who studied aspects of glutamic acid and GABA metabolism and kinetics. The study of De Krom (CLEO A 30) whose investigation of rectal application of Clobazam was in line with the studies on the pharmacokinetics of anti-epileptic drugs and of Bruens (CLEO A 23) who tested the usefulness of a portable EEG device for the ambulatory monitoring of seizures.

What has been the outcome of all these studies? In the course of this meeting a number of studies will be extensively discussed. The measurement of serum levels of anti-epileptic drugs has been a development which already bears its fruit for the present generation of people with epilepsy. Though not based on rigorous research, a number of authors felt confident to state that the improved prognosis with respect to seizure control as witnessed in recent years has to be credited to the possibility to monitor pharmacotherapy with the help of serum level measurements.

Paradoxically the most recent findings on the pharmacokinetics of anti-epileptic drugs show that, contrary to expectation, 24 hours profiles of serum levels cannot be predicted from knowledge of the half life of the drug and measurement of one serum level, preferably immediately before the next dose. Furthermore it has become clear that in the case of at least one of the currently most frequently used anti-epileptic drugs the relation between

serum level and effect is anything but simple.
I am referring to Valproate. One of our papers of years back is still often quoted (Meinardi et al. 1974). In it we presented evidence that for most patients who had responded well to Valproate therapy the serum levels recorded were close to 80 mg/L. In the meantime Binnie pointed out that measurement of the photoconvulsive threshold in photosensitive patients is an excellent instrument for fast determination of the anti-epileptic efficacy of a drug. Valproate was tested in this model. It does indeed show an elevation of the photoconvulsive threshold. It is, however, striking that this effect is still present after a return of the serum level of Valproate to zero. In this respect Valproate is clearly distinguishable from Progabide, with wich there is a direct correlation between serum level and increase of the threshold. The dissociation between the serum level of Valproate and the increase of the photoconvulsive threshold is also an argument against the hypotheses that Valproate exercises its anti-epileptic effect through an increase of gamma-aminobutyric acid levels (GABA) in the brain, an increase which, at least in animals, appears to run in parallel with the serum levels of valproate.

If we look at the practical application of serum level determinations for monitoring of people with epilepsy, we notice that in general one level is measured on a particular day and a next one after a few days or weeks, at least in the case of outpatient care. One would expect that a sample would be taken on the day that increase in dosage was decided upon and at the visit immediately following this change. This strict procedure is not always practised. Also when the patient is on constant medication periodic measurement of the serum level is indicated, as for several reasons it may show a gradual increase. This increase can be so slow as to allow the brain to adapt partially to the side effects, thus omitting warning symptoms. This effect has been well documented in phenobarbital. On the other hand such periodic tests can help monitoring patient compliance with the prescribed regimen.

It has been argued that measurement of serum levels of anti-epileptic drugs on an outpatient basis is useless, because the patient will come to the clinic at different times of the day and thus the serum level would refer each time to another part of the 24-hrs curve. In practice the situation is not all that bad. When we reviewed the laboratory data of patients who had been on constant therapy for over one year, either because they were seizure-free, or because the most optimal control of seizures had been reached, it turned out that the Carbamazepine level in 80% of the subsequent checks did not vary over 20%. In the case of

Valproate the picture was less favourable, but even there 51% of all measurements did not vary more than 20%. Perhaps it is difficult to give proof of the significance of serum level estimations for the every day care of patients with epilepsy.
This methodology has however beyond doubt proved itself for clinical trials of anti-epileptic drugs.
Classical examples are the studies of the assumed anti-epileptic activity of albutoin and hydantoyl valarianate (neo-citrullamon). Neither of these compounds appeared to be present in the serum of patients who several times had responded favourably to the drug. This also underlines the need for (placebo) controlled clinical trials. It has been proposed to make serum level determinations compulsory for clinical trials. This, however, is too rigid a point of view. For example one of the drugs that is presently investigated is pentyglycine. This molecule has been shown in animal experiments to pass the blood brain barrier rapidly. It is also quickly hydrolysed in pentanoic acid and glycine, of which an appreciable quantity is present in serum. Neither of them, however, can be easily used to monitor the drug.

The study of new anti-epileptic drugs is usually conducted without involvement of CLEO as CLEO is primarily an apparatus to finance research on epilepsy and secondarily concerned with coordination of this research. Unfortunately the financial stimulus by the government to channel research money through TNO (Netherlands Organisation of Applied Research) has decreased.
If not, then it would have been feasible to conduct the finances needed for clinical trials of anti-epileptic drugs through CLEO and use the extra amount for independent research.

Though the experience with clinical trials of anti-epileptic drugs now for more than fifteen years has encompassed the method of efficacy, assessment is still not completely satisfactory. Part of the problem is the lack of data prior to the clinical trial. It is desirable to have information on possible interactions with other drugs, which have to be used concomittantly, to be informed on possible seizure provocation due to drug withdrawal, on a possible time lag of the effect or a possible after-effect, when according to serum measurements the drug itself is no longer present. This information should determine the exact protocol of the clinical trial. In most clinical trials there is an instruction with respect to the study of side-effects. Nevertheless the study of the effect to these drugs on cognitive function usually is not spelled out in detail.

Aldenkamp, Alpherts and Moerland (CLEO A 52) are engaged in an analysis of the best battery of psychological tests which should be incorporated in clinical trials on anti-epileptic drugs.

In recent years people with epilepsy have been able to profit from important developments with respect to long-term EEG monitoring. Especially the techniques of ambulatory monitoring have increased diagnostic possibilities. In The Netherlands Binnie and coworkers have made important contributions in this field (CLEO A 38). They have also applied this technique to study the influence on brain activity, as manifested in the EEG, of behavior (CLEO A 51). In particular transient cognitive impairments have received their attention. These studies have revived the question whether one should abstain from treatment if the EEG gives evidence of irritative activity while clinical observation does not succeed in detecting clinical seizures. In other words is it possible to perform EEG cosmetics without improving functioning of the patient or are epileptiform changes in the EEG pattern always indicative of demonstrable seizure activity if the diagnostic methods are of sufficient sensitivity?

Let us summarize once more the effect of 55 CLEO grants. The finding of a genetic marker for primary generalized epilepsy did not succeed. In this respect Delgado-Escueta in the United States seems to be more successful with respect to the Janz syndrome (1984).

Our knowledge of the pharmacokinetics of anti-epileptic drugs has increased tremendously, even to the extent that we now have to ask ourselves whether serum level sampling is an appropriate tool to assist in rational pharmacotherapy.
To illustrate this point I would like to remind you of the study of 48-hours profiles of Carbamazepine levels which showed roughly three types of circadian curves: the flat curve, the early morning peak and the rise curve. Often one and the same patient would have a different type of curve on two consecutive days. I would also like to remind you of the discrepancy between the short half life of Valproate in serum and its prolonged action which can be so beautifully demonstrated by the suppression of photosensitivity in patients with a photoconvulsive response.
Though I mentioned the important progress in the diagnostic use of electroencephalography our goals have not all been met. For example one of CLEO's grants was concerned with the development of telephone telemetry in order to achieve twenty channel EEG recordings at the patient's home or at his work. This system is waiting for adequate technical support. However, the expectation that mobile cassette recording would make telephone telemetry obsolete has not been fulfilled either.
Sociological studies have not given other clues to assist people with epilepsy than through the provision of adequate information. Theoretically parents should be informed about the prognosis for their child. The question of prognosis is not only at stake at

the first moment the diagnosis of epilepsy is made. The question becomes even more cogent when the patient has not had seizures for a while and the choice for his future career has to be made.

With how much certainty can it be predicted that seizures will stay away and which factors can perturb the freedom of seizures? The medical adviser of the nautical inspector is of the opinion that stress on board ships is such that a patient who is free of seizures can thus get a relapse. Obviously he has no proof for that assumption. Unfortunately there is also no proof to the contrary.

Another problem that needs attention is whether experience with the issuing of drivers licences to patients who have been free of seizures for over two years would not provide material to decide whether the restrictive policies with respect to driving lorries and public transport vehicles should not be revised.
All past years of research have still not clarified whether learning problems of children with epilepsy are reversible, nor has it become clear if understanding the pathophysiology of the epileptic process will be helpful in remedial teaching.

I was invited to speak about two aspects today. The first aspect was a review of what has been achieved. The second was to predict what could be done in the future.
For prognostication different techniques can be used. One is based on analysing ongoing developments and predicting their outcome. Another is based on extrapolation of the course of events in the past. Especially the second technique can easily be disturbed by the change of extraneous forces of which the impact on historic events was not recognised to begin with. For example the development of telephone telemetry stranded on the closure of the Institute for Medical Physics TNO. It's occurrence was not foreseen when the project was started as the influence of changes in TNO management was not recognised to be of any importance for the realisation of this project.
When the future of care for people with epilepsy is looked at, often the example of care for people with tuberculosis is quoted, with the sudden drastic decrease of the need for sanatoria and consultation clinics. Though the discovery of a perfect anti-epileptic drug cannot be excluded, nevertheless the process of epileptogenesis seems to be so much multifactorially determined that a comparison between chemotherapy of tubercle baccilli and normalisation of brain function in epilepsy is not realistic.

Technological developments seem easier to predict. For example one may anticipate that further developments of magneto-encephalography are apt to make electroencephalography, even with

its extension of intracerebral recording, partly obsolete. Our knowledge about the events leading to sudden excessive discharges of neurones will no doubt increase tremendously through the use of positron emission tomography and magnetic nuclear resonance.

Special centres for epilepsy may have to disappear not because brain function of people with epilepsy can be more readily normalised, but because one of their major goals, the observation of the patient with epilepsy, can be reached at home. Both the increasing amount of leisure time in society which will allow relatives to stay home and look after the ill, and also the technological developments like continuous recording of the EEG at home with video recording of the seizures by one of the family will do away with the need for hospitalisation for seizure analysis.
Of course for this purpose further miniaturization of techniques as magneto-encephalography and improvement of automatic data analysis are required.
Special centres for epilepsy also provide the means to learn how to live with epilepsy. This function of the centres is much less easily transferred to the home situation.

The present intervention techniques make use of interactions between persons with similar problems. In The Netherlands it is possible to use this technique on groups of sufficient size and homogenity by concentrating people with severe epilepsy problems in three special centres. Treatment at home will require different intervention techniques which may well be an utopia.
With respect to the decentralisation of the care for people with epilepsy I have postulated at another occasion that this may be enhanced by the development of computerized expert systems which will assist the general practitioner, who is seeing too few patients with epilepsy to develop sufficient know-how, to treat his patients according to the best knowledge available. Such a system is only of use when the results of each treatment are reported to a control-post where all data can be analysed and the computer programms subsequently be adjusted.

The prevention of epilepsy is not a major undertaking. Obviously there are more reasons to prevent brain damage than just the wish to reduce the incidence of epilepsy. It must be realised that 5% of all brain damage and 15% of severe brain damage is followed by the development of epilepsy. To prevent such damage in the first place is the concern of neurologists and the medical profession as a whole.
Similarly the prevention of birth trauma or intracranial infections is not a frontline where epileptologists can join the battle. This is different regarding the question of reducing the

disposition for seizures. The pseudo-eugenetic excesses of the Third Reich for many years have made research in the genetics of epilepsy suspicious.

Transmission of epilespy has such a low incidence that the choice of a partner will not easily be influenced by the fear to get a child with epilepsy. Our knowledge about the heredity of epilepsy may start to bear fruit when gen modification techniques are more developed. The present state of the art is still at te level of the drosophila. For mankind probably the first disease amenable to genetic engineering will be those with a defect in bonemarrow cells like the Lesch-Nyhan syndrome (deficit of hypoxanthine-guanine phosphoribosyl transferase) and certain immunodeficiency syndromes like the lack of purine nucleoside phosphorylase and the lack of adenosine deaminase.

Genetic studies in epileptology would also be concerned with pharmacogenetics. Over every user of anti-epileptic drugs a sword of Damocles is dangling which, when it falls, will bring about an idiosyncratic drug reaction. This comprises a broad spectrum in which now one than another organ and often several are involved. Perhaps birth defects which are seen in 10% of children exposed in utero to anti-epileptic drugs belong to this class of idiosyncratic reactions and may be prevented if more was known about the inheritance of these factors.
The ideas presented are only partly extrapolations of the developments taken place in the past. If extrapolations were to be based only on past events, these whould be suspect as it is unlikely that modifiers that were active in the past will remain the same in the future.
For example genetic engineering will produce a completely new setting, the effects of which cannot yet be predicted, because our knowledge of the heredity of epilepsy is too scanty. With these developments in mind time has come to increase our efforts in the fields pioneered by Janz, Doose, Delgado-Escueta and Noebels. The impact of brain tissue transplantation has not even been discussed yet. This technique leaves ample room for speculations.
Science is the art of predicting events that can be falsified or verified. Predictions that as yet escape testing belong to the realm of clairvoyants, astrologists and augurs. CLEO, at least her boss Apollo, favored the augurs. It is to be hoped that the "Commissie Landelijk Epilepsie Onderzoek" will continue to favor science.

References

Bongers, H., Osinga, I., Bongers, E. en Meinardi, H. Kö 1173 - Mexiletine - in the control of epileptic seizures; a double blind cross-over trial versus placebo. In: M. Dam (Ed.) Controlled trials of anti-epileptic drugs. Proceedings of the International Workshop on controlled trials of anti-epileptic drugs, 1974.

Delgado-Escueta, A.V. en Greenberg, D. The search for epilepsies ideal for clinical and molecular genetic studies. Ann. Neurol. 1984, 16 (suppl).: 1-11.

Gardner-Thorpe, C., Janz, D. Meinardi, H. en Pippenger, C.E.: Anti-epileptic drug monitoring. Proceedings of the Workshop on the determination of anti-epileptic drugs in body fluids III, 1976, 388 pp.

Houtkooper, M.A., Oorschot, C.A.E.H. van, Rentmeester, T.W., Höppener, P.J.E.A. and Onkelinx, C. Double blind study of Milacemide in hospitalized therapy-resistant patients with epilepsy. Epilepsia (In press).

Johannessen, S.I., Morselli, P.L., Pippenger, C.E., Richens, A., Schmidt, D. en Meinardi, H.: Anti-epileptic therapy: Advances in drug monitoring. Proceedings of the Workshop on the determination of anti-epileptic drugs in body fluids, 1979, 425 pp.

Kulig, B.M., Meinardi, H. en Stores, G.: Epilepsy and Behavior. Proceedings of the Workshop on the Psychological Assessment of persons with epilepsy, 1980, 203 pp.

Levy, R.H. Pitlick, W.H., Eichelbaum, M. en Meijer, J.W.A.: Metabolism of anti-epileptic drugs. Proceedings of the workshop on the metabolism of anti-epileptic drugs, 1982, 245 pp.

Linden, G.J. van der, Meinardi, H., Meijer, J.W.A., Bossi, L. and Gomeni, C. A double blind cross-over trial with Progabide (SL 76002) against placebo in patients with secondary generalized epilepsy. In: M. Dam, L. Gram en J.K. Penry (Eds.), Advances in Epileptology: XIIth Epilepsy International Symposium, 1980: 141-143.

Lindhout, D. Teratogenesis in maternal epilepsy; new aspects of prevention. Thesis. Amsterdam Free University, 1985.

Mandel, P. Tactical considerations and practical achievement in research for new anticonvulsant drugs. In: H. Meinardi and A.J. Rowan (eds.), Advances in epileptology, 13th Congress of the Internationa League against epilepsy and 9th Symposium of the International Bureau for Epilepsy, 1977: 156-157.

Meijer, J.W.A., Meinardi, H., Garner-Thorpe, C. en Van der Kleijn, E. Methods of analysis of anti-epileptic drugs. Proceedings of the Workshop on the determination of anti-epileptic drugs in body fluids, 1972, 257 pp.

Meinardi, H. Clinical trials of anti-epileptic drugs. Psychiat. Neurol. Neurochir. 1971, 74: 141-151.
Meinardi, H., Hanke, N.F.J. en Beveren, J. van: Sodium di-n-propylacetate: estimation of effective serum levels. Pharmaceutysch Weekblad, 1974, 109, nr.2: 45-47.
Melman, A.P.M. Verandering van botmassa door exogene factoren. Thesis 1978. Leyden University.
Millichap, Gordon J. and Ulrich, J.A. Abnormal urinary excretion of amino acids in children with petit mal epilepsy: Preliminary Communication. Proceedings The Mayo Clinic, 1962, 37: 307-310.
Reynolds, E.H. Mental effects of antiepileptic medication: a review. Epilepsia, 1983, 24, suppl. 2: 85-95.
Schneider, H., Janz, D., Gardner-Thorpe, C. Meinardi, H. and Sherwin, A.L. Clinical pharmacology of anti-epileptic drugs. Proceedings of the Workshop on the determination of anti-epileptic drugs in body fluids II, 1974, 370 pp.
Starreveld-Zimmerman, A.A.E., Kolk, W.J. van der, Elshove, J. and Meinardi, H. Teratogenicity of anti-epileptic drugs. Clin. Neurol. Neurosurg., 1974, 77-2: 81-95.
Suurmeijer, T.P.B.M. Kinderen met epilepsie; een onderzoek naar de invloed van ziekte op kind en gezin. Thesis, Groningen University, 1980.

POSTERS

ENDOGENOUS OPIOID PEPTIDES AND EPILEPSY.

J. Haffmans, O.E. Ukponmwan and M.R. Dzoljic

Many reports including those from our laboratory, indicate that intracerebroventricular (ivc) administration of enkephalins, endorphins or enkephalinase inhibitors, which block the biotransformation of enkephalins, result in epileptiform discharges. The enkephalins are released during kindled and convulsive seizures. Postictal seizure suppression can be reduced by pretreatment with naloxone. Therefore, it has been suggested that opioid peptides may play a role in the appearance of certain postictal and interictal phenomena including postictal suppression of subsequent convulsive seizures.
In this study, we were mainly concentrated on further indentification of the type op opiate receptors, brain regions and modulating factors involved in the opioid-induced excitatory phenomena.

The experiments suggest that the short onset of enkephalin-induced excitatory phenomena, after icv administration in rat, is due to the rapid distribution and penetration of the enkephalin in the surrounding periventricular tissue (Haffmans et al. 1983). By using selective agonists for the μ and δ opiate receptors respectively, it has been demonstrated that the epileptiform activity of the opioid peptides is a result of an activation of the δ opiate receptors in the CNS (Haffmans and Dzoljic 1983). The recently described δ opiate receptor antagonist, ICI 174,864 antagonized the DSTLE-induced epileptiform EEG spike activity (thesis, J. Haffmans). It is proposed that the subiculum or the pyramidal cells of the CAI hippocampal area, are the brain regions the most involved in the enkephalin-induced seizure phenomena. This is supported by electrophysiological recordings, data concerning the metabolic rate and the regional cerebral blood flow in the hippocampal regions during enkephalin-induced excitations (Haffmans et al. 1984; Haffmans et al. 1985).

It is known that the endorphinergic system modulates the release and, activity of several transmitters. We showed that other systems might affect the endorphinergic system as well. Activation of the MAO-B system may significantly increase the seizure activity induced by enkephalins (Ukponmwan et al. 1983). This might be of relevance in clinical situations where an altered MAO-B system (psychosis, stress, anti-depressive therapy) may significantly modulate the neuronal excitability by altering the activity of the endogenous opioid peptides.

REM sleep deprivation (REMSD) increases neuronal excitability and facilitates seizure activity. The mechanisms underlying this action is not yet understood. In our experiments, REMSD potentiated enkephalin-induced seizures and increased the frequency of handling induced convulsions (HIC) in mice. This effect of REMSD on HIC was abolished by phosphoramidon, an enkephalinase inhibitor which increases the enkephalin concentration in brain. The effect of phosphoramidon was abolished by naloxone. Therefore it is suggested that REMSD is associated with an insufficiency of the enkephalinergic system (Ukponmwan and Dzoljic 1984). It is known that endogenous opioids exert an inhibitory action on the release of excitatory neurotransmitters. This might indicate that not only activation but also insufficiency of this system may increase neuronal excitability. An insufficiency of the endorphinergic system, as a result of REMSD, might be of importance for the clarification of the worsening of seizures or improvement of depressive disorders in patients during REMSD. The known physiological changes such as circadian rhythms in the endorphin release or the increase of endorphin concentration with age, might also be of importance for the modulation of the neuronal excitability. This might be of clinical relevance for patients whose attacks are affected by stress, age or sleep-waking cycle.

References

Haffmans, J., Blankwater, Y.J., Ukponmwan, O.E., Zijlstra, F.J., Vincent, J.E., Hespe, W., Dzoljic, M.R., Neuropharmacology 1983, 22: 1021-1028.

Haffmans, J., Dzoljic, M.R., J. Neural. Transm. 1983, 57: 1-11.

Haffmans, J., De Kloet, R., Dzoljic, M.R., Brain Res. 1984, 302: 111-115.

Haffmans, J. Heiligers, J., Dzoljic, M.R., Saxena, P.R., 1985 (in press).

Ukponmwan, O.E., Van der Poel-Heisterkamp., A.L., Haffmans, J., Dzoljic, M.R., Naun.-Schmied Arch. Pharmacol. 1983, 322: 38-41.

Ukponmwan, O.E., Dzoljic, M.R., Psychopharmacology, 1984, 83: 229-232.

NEUROPSYCHOLOGICAL ASSESSMENT IN EPILEPSY:
demonstration of a computerized test battery.

A.P. Aldenkamp, W.C.J. Alpherts en M.C. Moerland

In clinical treatment of people with epilepsy classical psychometric assessment seems to run short when questions arise about specific mental (sub)functions, e.g. in evaluating the effects of

anticonvulsant drugs, the influence of seizures and focus of seizures on mental abilities, finding mental correlates of EEG measures, detecting deterioration, planning neurosurgery, etc.

In recent years cognitive research and neuropsychology gave a new impetus to testing practice. It is from these two lines of thought that we are currently developing a comprehensive testbattery, which seems to cope with the problems mentioned before. The battery roughly covers the following areas:
1. Memory functioning
2. Attention and concentration
3. Central cognitive processes
4. Flexibility and tempo
5. Language functioning
6. Perceptual and motor functions.

Computerized forms of existing tests as well as new experimental tasks have been developed. In the memory area differential assessment of sensory store, short term memory, long term memory and remote memory is now possible for both verbal and non verbal material and for different input and output modalities. Data on reliability and validity of the different subtests as well as normative data for 'epilepsy', 'normal' and 'brain damaged' adults will be sampled. Our current focus is at attention and concentration processes. Computerized tests from different areas (memory, attention, etc.) will be demonstrated.

THE DIAGNOSIS OF EPILEPSY BY MEANS OF SLEEP RECORDINGS.

A.C. Declerck, W.L.J. Martens, C. Sijben-Kiggen

For several decades polygraphic sleep recording has been carried out to confirm the existence of epilepsy and to better describe its form and the spread of epileptiform activity in the brain. Based on our own investigations the usefulness of this method will be shown.

During the period of 1976-1981 1503 patients, which had or were suspected to have epilepsy, were investigated by means of long-term polygraphic sleep records. They consisted of all night recording or 3-4 h sleep recording following one night of total sleep deprivation. All registrations were made by means of a 16 or 21-channel electroencephalograph and visual description and semi-automatic analysis was afterwards performed. Sleep as well as epileptic EEG phenomena were evaluated and registered in a standardized way.

Our investigations resulted in answers to a number of questions, including the following:

1. what is the diagnostic gain for the different forms of epilepsy, depending on the kind and depth of sleep?
2. is the degree by which the existence of epilepsy can be confirmed or denied depending on the clinical probability of having epilepsy?
3. is the possibility to diagnose epilepsy larger by increasing the duration of the recording?

We may conclude that:

1. polygraphic sleep recording is an important resource for the diagnosis of epilepsy;
2. in this respect, sleep recording following one night total sleep deprivation is a very useful method;
3. the diagnostic confirmation of epilepsy increased with 13% following a recording of two sleeps cycles (about 4 h) in contrast with recording of one sleep cycle (about 2 h).

DIAGNOSIS OF EPILEPSY: LONG-TERM SLEEP RECORDING VERSUS 24 H AMBULANT EEG RECORDING.

A.C. Declerck, P.H.M. v.d. Ham-Veltman, E. Barten

To confirm or to better describe the existence of epilepsy in some difficult cases, a routine EEG can be supplemented by a polygraphic sleep recording or by an ambulant long-term EEG recording. In spite of the fact that these methods differ substantially from each other, there are no obvious criteria to prefer either of them. We investigated this problem in 100 patients which had or were suspected to have epilepsy. Apart from a routine EEG recording, a 3-4 h sleep recording following one night of total sleep deprivation and a 24-36 h ambulant EEG recording were carried out. Sleep recordings were made using a 16-21. channels electroencephalograph and the ambulant EEG registrations were performed with a 4-channel system. Selection of the localization of the electrodes was based on clinical and anamnestic data and results from earlier EEG findings. All EEG recordings were carried out within a period of one week during which treatment was unchanged.

The patient population was subdivided into two categories: one in which there was a high probability of epilepsy and one where the diagnosis of epilepsy was doubtful. For both categories and for the three different forms of recordings, we calculated the number of registrations in which epileptic EEG activity was present.

The main conclusions from our study are:
1. As compared to a routine EEG recording, both methods provide a comparable diagnostic improvement.
2. In doubtful cases it is useful to apply both methods since in 10% of the population the diagnosis could only be established with either one of these methods.

THE DIAGNOSTIC VALUE OF GENERALIZED SPIKE-AND-SLOW-WAVE PAROXYSMS DURING WAKEFULNESS AND SLEEP.

A.C. Declerck, P.H.M. v.d. Ham-Veltman, M. te Dorsthorst

Some information is now available on the influence of sleep on epilepsy. The way and the degree by which spike-and-slow-wave paroxysms change during sleep and wakefulness and the significance of these changes with respect to the diagnosis of epilepsy is far less known. This problem was studied in 300 patients. They were selected from a population of 2500 because: 1. they had a well-known form of epilepsy; 2. a polygraphic sleep recording was performed in all of them. The following groups were studied: 1. 100 of them had a 3 c/sec spike-and-slow-wave paroxysm during their awake EEG; 2. 100 had a low frequency spike-and-slow-wave paroxysm (less or equal than 2.5 c/sec); 3. 100 patients had polyspike and slow-wave paroxysms (2 or more spikes). For each of these three groups we investigated in which form the epileptic manifestations appeared during light and deep NREM sleep. Thereafter, we investigated the correlation between the different paroxysms and the clinical form of epilepsy, independent of the condition of sleep or wakefulness during which they were recorded. Our investigation demonstrated that the electroencephalographer needs to be aware of the degree and the way in which spike-and-slow-wave paroxysms change during wakefulness and sleep and its diagnostic implications, in order to avoid misinterpretations.

FOOD INTAKE AND ANTIEPILEPTIC DRUGS. EVIDENCE FOR A ROLE OF GABA IN THE CIRCADIAN TIME KEEPING.

W.J. Rietveld, K. Schravendijk

The hypothalamic suprachiasmatic nucleus (SCN) plays an important role in the mechanism of circadian time keeping. The SCN in most mammals is a densely populated area just above the optic chiasm receiving visual information via a direct retino-hypothalamic

projection, (RHP). There is a vast amount of evidence that the SCN are acting as a major circadian pacemaker, entrained to the 24 hour alternation of light and darkness by the RHP. However, less is known about the way the circadian signal is generated within the SCN. Immunohistochemical methods have revealed numerous neuropeptides, transmitters as well as modulators, located within different cells of the SCN. So more recently studies are carried out using a neuropharmacological approach.

Data from literature indicate that sodium-valproate, its precursor dipropylacetamide as well as carbamazepine are clinically effective in the treatment of bipolar depressions. These drugs share the property of enhancing the GABA mediated inhibition be it through rather various mechanisms. As it is known that antiepileptics are able to affect the circadian system as well as that the SCN contain a rather numerous amount of GABA positive neurones, we became interested in the effect of long term application of sodium-valproate on the circadian control of behavior.

Continuous and automatic recording of food intake of the rat was chosen as the rhythm to study, whereas the sodium-valproate was applied through the drinking water. In four out of six animals it was possible to increase the dose up to 1000 mg/kg body weight/day. The two other rats decreased their water intake according to the dose so that they never reached a level higher than 750 mg/kg/day. The four animals showed a shortening of the period from about 24.30 to 24.10 h. The activity/rest ratio showed an increase from about 1.0 to 1.5. So, especially at higher dose levels the pattern of food intake changed. After termination of the valproate all animals showed an almost immediate lengthening of their period, returning to values comparable to those before application of sodium-valproate, (see Figure). These results suggest a role of GABA-ergic cells in the generation of the circadian pacemaker signal.

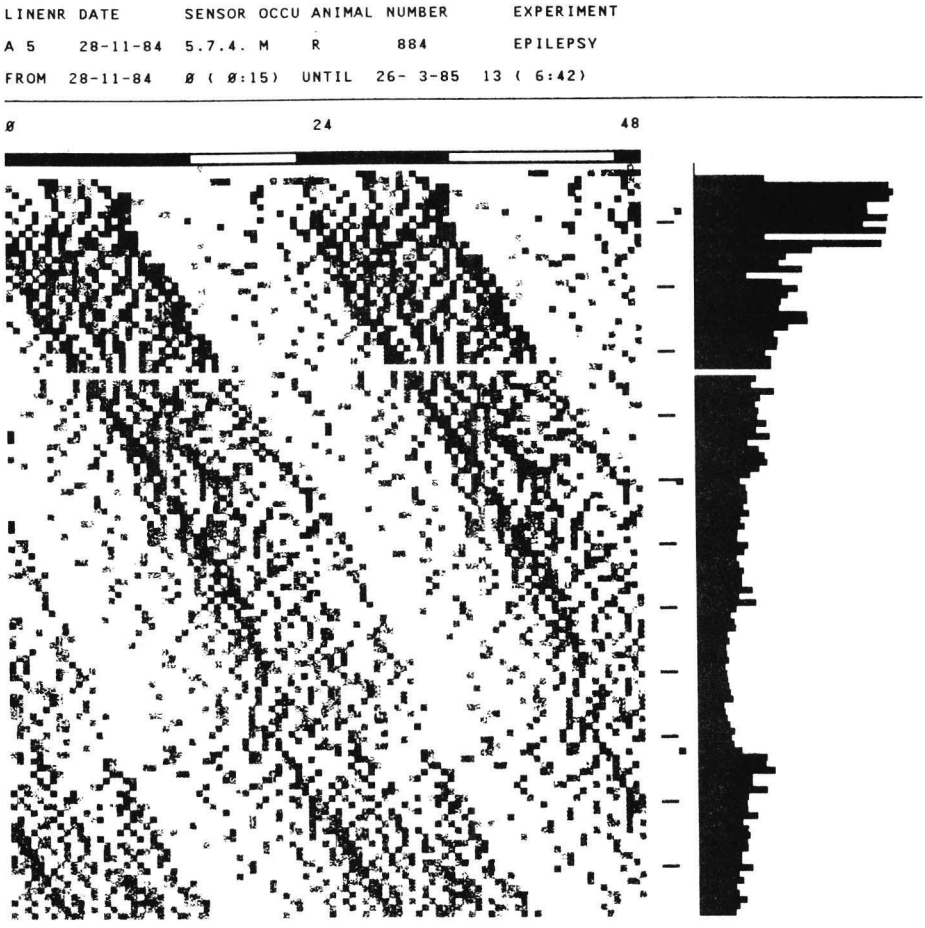


Figure: Plot of food intake of a male Wistar rat before, during and after oral administration of sodium valproate. The amount of food is plotted in half hourly intervals, successive days are plotted in the vertical axis. For reasons of visual display a double plot is made in which two successive days are plotted after each other. The bars on the right hand side mark ten days intervals. The dark squares between the bars mark the day of blinding, the beginning and the end of the sodium valproate application respectively. The black band at the utmost right hand side indicates the amount of food eaten during 24 hours.

EPILEPSY AND HEALTH EDUCATION, INFORMATION PROCESSING AND EXPERIENCE.

J. Gutteling, E.R. Seydel

The existence of wrong ideas about epilepsy influences the functioning of people with epilepsy, either directly or indirectly (De Boer 1984; Höppener et al. 1984). Health education is seen as a possibility to alter wrong ideas. Numerous health education methods and programs have been developed and some of them evaluated. Conclusions on the effectiveness of these programs are hardly possible, due to some methodological and conceptual fallacies (Gatherer et al. 1979). This is also the case with health education about epilepsy (Rose et al. 1955; Sands and Zalkind 1972; Matovu 1974). To avoid content and method confusions in our study methods are manipulated independent from content. In all methods exactly the same information was used, only the method of education was varied, from a relatively simple leaflet to a more complex and intensive group discussion with audio-visual support.

Both Fishbein and Ajzen's theory of reasoned action (Fishbein and Azjen 1975) and the Health Belief Model (Becker et al. 1977) stress the importance of experiences in the process of attitude formation. Regan and Fazio (1977) and Fazio and Zanna (1981) state that the kind of experiences (behavioral or non-behavioral) determines the consistency between attitudes and behavior and influences the effectiveness of new information.

On account of this the following research questions were raised:
What is the effect of:
. the different health educational methods?
. experiences with epilepsy?

This field-experimental study represents a control group design with a pretest and 2 posttests, the independent variable being Health Education method. Subjects were 132 students (42% male, 58% female) from 3 teacher-training colleges, ranging in age from 20 to 25 years.

Based primarily on existing material, used by the Dutch Federation against Epilepsy, and on theoretical notions (Van der Ban 1982) the following three educational methods were used:
. Leaflet method with information about epilepsy.
. Audio-visual method consisting of 20 colour slides with accompanying text.
. Group discussion method. Following a brief instruction by the health educator the participants watched the audiovisual and thereafter took part in a group discussion about epilepsy.

Results

- In this study we found an increase in knowledge and attitude change directly following the health education and a long-term increase of knowledge.
- We could not confirm the superiority of one educational method over the others; in general the effects of the various methods did not differ.
- The opinion of the participants about the group discussion method and the role of the health educator are significantly more positive than both other methods.
- Level of experience with epilepsy appears to interfere with the increase of knowledge and attitude change. This can be concluded from the result that individuals with a lot of experience with epilepsy and a high level of knowledge before the health education were influenced to a lesser extent than individuals who had less experience with epilepsy and knew less about it.

Conclusions

It seems unimportant which educational method one chooses in the case of epilepsy. However, the health educator should bear in mind the informational needs and characteristics of his target group.
The conclusion is that the health educator should consider the experience factor because it seems that a lot of experience with epilepsy can restrain the processing of new information and attitude change.

References

Ban, A.W. van der, Inleiding in de voorlichtingskunde, Boom, Meppel, The Netherlands, 1982.

Becker, M.H. et al. Selected psychosocial models and correlates of individual health related behaviors. Medical Care, 1977, 15: 27-46.

De Boer, H.M. Sluit epilepsie bepaalde beroepen uit? Adviezen aan de bedrijfsarts; In: A.E.H. Sonnen (Ed.), Epilepsie en Risico, Supplement Epilepsie Bulletin, 1984.

Fazio, R.H. and Zanna M.P. Direct experience and attitude-behavior consistency. In: L. Berkowitz (Ed.) Advances in experimental social psychology Vol. 14. Academic Press, New York, 1981.

Fishbein, M. and Ajzen I. Belief, attitude, intention and beha vior. An introduction to theory and research. Addison Wesley, Read. Mass., 1975

Gatherer A. et al. Is health education effective? The Health Education Council, London, 1979.
Höppener, R. et al. Hebben mensen met epilepsie problemen met het afsluiten van verzekeringen? In: A.E.H Sonnen (Ed.) Epilepsie en Risico, Supplement Epilepsie Bulletin, 1984.
Matovu, H.L. Changing community attitudes toward epilepsy in Uganda. Social Science & Medicine, 1974, 8: 47-50.
Regan, D.T. and Fazio, R.H. On the consistency between attitudes and behavior: look to the method of attitude formation. Journal of experimental social psychology, 1977, 13: 38-45.
Rose, A.W. et al. On changing social conceptions of epilepsy. Epilepsia, 1955, 4: 99-107.
Sands, H. and Zalkind S.S. Effects of an educational campaign to change employer attitudes toward hiring epileptics. Epilepsia, 1972, 13: 87-96.

VALUE OF POSTICTAL PROLACTINE STUDIES FOR DIFFERENTAL DIAGNOSIS IN EPILEPSY.

W. van Emde Boas, E. Endert

Postictal prolactine (PRL) elevation after epileptic seizures has repeatedly been reported and use of PRL for differential diagnosis between real and pseudo-seizures has been proposed.
Published findings are inconsistent in some types of seizures concerning maximum postictal PRL values. Physiological PRL fluctuations are consistently ignored, which may contribute to clinical misinterpretation.

Spontaneous and postictal PRL fluctuations during 24-48 hours were studied in 15 patients with various types of epilepsy under continuous EEG and video monitoring. Blood samples were collected at 60 min. intervals through an indwelling catheter; additional postictal samples were collected in 3 patients.
8 Patients showed one or more seizures during monitoring; a pseudoseizure was recorded in 1 patient.

Results show that postictal PRL peaks can remain within physiological limits, even after generalized fits.
After partial seizures PRL peaks are inconsistent and may not exceed 2-3 times preictal values; comparable fluctuations can be found after pseudoseizures or emotional stress.
PRL peaks within physiological limits may have clinical or diagnostic significance in epileptology, but knowledge of a patient's spontaneous PRL profile is necessary for reliable interpretation. Routine use of PRL for differential diagnosis of seizures thus seems of limited value, especially in the care of partial seizures.

CLINCAL VALUE OF WHOLE NIGHT SLEEP MONITORING OF SUSPECTED ICTAL EVENTS IN CHILDREN.

W. van Emde Boas, C.D. Binnie, J. Overweg, A. van Wieringen

Episodic disturbed nocturnal behavior in children is common. For differential diagnosis between epileptic and non-epileptic phenomena the child may be referred for whole night sleep EEG and video monitoring, which is expensive and time-consuming. To evaluate the actual clinical value of this method for the referring physician all whole night EEG and video-recording studies of children, performed in our Institute over 1978-1982 were reviewed.

95 Registrations were obtained form 66 children. Of these, 55 studies in 45 children aged 1 to 14 years addressed specific questions regarding nocturnal behavior disturbances. 35 Of these studies were preceded by elaborate clinical observations. In 28 children epilepsy had been established; in the other 17 epilepsy was considered possible. All recordings were made using standard 16 or 20-channel cabletelemetry EEG equipment with continuous video-monitoring.

Indications for monitoring were mainly suspected seizures (N=22), motor behavior of uncertain origin (N=18) and enuresis nocturna (N=11). Clinical questions concerned mostly the epileptic origin (N=36), the documentation and frequency of the events (N=16), or the absence of seizures (N=4). 12 Registrations were requested in part to solve a disagreement between parents and nursing staff about the presence or significance of certain nocturnal phenomena.

Intensive sleep monitoring provided a definite answer in 67.2% of all registrations and was strongly suggestive in 5.5%. A partial answer was possible in 10.9%. In only 9 studies (16.4%) no answer was possible. Clinical observation, although insufficient to solve the problem was valuable in identifying patients worth monitoring. Nocturnal events were more often present in in-patient studies than in out-patients and results in in-patients were far better (87.7% complete or almost complete answers) than in out-patients (50%).

In 22 studies for seizures real epileptic seizures were seen in 12. A complete answer was possible in 72.2% of the studies. Nocturnal restlessness was seen in 13 out of 18 studies but was epileptic in only 3. A complete answer was possible in 62% of the studies where epilepsy was possible and in 70% in those where

epilepsy was certain. Enuresis was present in only 3 out of 7 studies in epileptic children and only once followed an epileptic seizure. In this group an answer was possible in only 42.9% of the studies. Results were better in the group without certain epilepsy, yet overall effectivity in enuresis was only 63.6%. In all cases of discrepancy between parental and nursing observations an objective verdict was provided. In epileptic children the parents proved right 7 out of 9 times in reporting seizures which were misinterpreted by the nursing staff. Intensive monitoring resulted in a change of diagnosis of classification of epilepsy in 16 studies (35.5%).

From these results it can be concluded that whole night sleep EEG and video-monitoring is a valuable method for investigating paroxysmal nocturnal behavior in children, answering the clinical questions of the referring physician in 72% or more of the cases. Seizure-like activity or excessive restlessness of uncertain origin are good indications. Monitoring exclusively for enuresis nocturna is to be discouraged. Clinical observation prior to monitoring will help to identify the right patients for these studies. The method is especially valuable in solving any disagreement between parents and professional observers about the presence or clinical significance of suspected nocturnal seizures and will help to improve mutual trust and confidence.

AUDIOGENIC SEIZURE SUSCEPTIBLE RATS: A NON-INVASIVE IN VIVO MODEL OF EPILEPSY.

L.H. Schrama, G.J.A. Ramakers, P.N.E. de Graan and W.H. Gispen

One of the major problems in studying the molecular mechanism of epileptogenesis is the lack of suitable experimental models. Recently we discovered that about 50% of the male population of our inbred Wistar strain is susceptible to high frequency auditory stimulations. These rats respond to the sound produced by an ultrasonic source with a typical sequence of behavioral elements: a startle response, freezing, violent running and jumping that may be terminated by a tonic-clonic seizure or repeat of the freezing, violent running, tonic-clonic seizure and postictal depression.

This behavior is recorded on videotape and analyzed in terms of the audiogenic response score (ARS). Furthermore, we study to what extent this behavior is frequency dependent and can be modulated by pharmaca (e.g. anti-epileptics).

This non-invasive in vivo model of epilepsy may be highly suitable to study biochemical correlates of epileptic behavior and will be used to determine the role of the phosphorylation of synaptic elements in the molecular mechanism of epileptogenesis.

THE 4-AMINOPYRIDINE MODEL OF EPILEPSY: Ca^{2+}/CALMODULIN-DEPENDENT CHANGES IN PROTEIN PHOSPHORYLATION.

L.H. Schrama, P.N.E. de Graan, G.J.A. Ramakers, F.H. Lopes da Silva and W.H. Gispen

Changes in protein phosphorylation have been shown to be highly correlated with long-term potentiation after application of a tetanus in rat hippocampal slices (Tielen et al. 1983). In this in vitro slice system the convulsant 4-aminopyridine (4-AP) is known to induce characteristic changes in electrophysiological properties of the neuronal membranes. Using this experimental model, we now studied a possible role of protein phosphorylation in epileptogenesis. After incubation of slices with or without 4-AP, a crude mitochondrial: synaptosomal fraction was prepared and assayed for endogenous protein phosphorylation using γ-^{32}P -ATP. The 4-AP increased the phosphorylation of a 50 kDa protein by more than 100%, whereas the phosphorylation of a protein of Mr 48 kDa (B-50) was decreased. No changes in phosphorylation were found in the 80, 52, and 43 kDa proteins. The 4-AP-induced effect was dose dependent (EC_{50}: 5 x 10^{-7}M) and detectable within minutes. Thus, the 4-AP concentrations required to induce effects on 50 kDa phosphorylation are in the same range as those required for electrophysiological changes. The in vitro 50 kDa protein phosphorylation is strongly calcium/calmodulin dependent. Therefore this 50 kDa protein strongly resembles the 50 kDa protein described by Wasterlain and Farber (1982) the phosphorylation of which is strongly affected by kindling in another experimental model of epilepsy. This protein is most likely the ρ/α-subunit of the calcium/calmodulin-dependent protein kinase (Bennett et al. 1983; Wasterlain and Farber 1984). Our results are consistent with the proposal that Ca^{2+} plays a crucial role in epileptogenesis and suggest the involvement of calcium/calmodulin-dependent phosphorylation in the 4-AP model of epilepsy.

References

Bennett et al., J. Biol. Chem. 258, 1983: 12735
Tielen et al., Brain Res. 277, 1983: 189
Wasterlain and Farber, Brain Res. 247, 1982: 191
Wasterlain and Farber, PNAS 81, 1984: 1253.

PARADOXICAL SLEEP DEPRIVATION AND TONIC AROUSAL PROCESSES IN THE RAT.

Z.J.M. van Hulzen, J.M.H.H. van Hest and A.M.L. Coenen

Deprivation of paradoxical sleep (PS) by instrumental or pharmacological means is generally followed by a "rebound" effect, in which the amount of PS can exceed far beyond normal levels. It would be interesting to know whether the PS deprived state is reflected neurophysiologically in the waking state. According to the neural excitability hypothesis of PS (Dement 1965), there is a generalized increase in the "excitability" of the central nervous system.

In a previous study (Van Hulzen and Coenen 1984) the P3-N3 amplitude of the photically evoked response in the visual cortex was measured in waking rats immediately following 72 hours of PS deprivation. One of three different techniques were employed to establish PS deprivation instrumentally. The P3-N3 amplitude was found to be decreased as a result of PS deprivation, which was interpreted as indicating an increase in tonic arousal having a depressing influence on visual cortical excitability (Steriade 1970). As a correlative finding, a gradual increase in the same measure was observed in the course of habituating to a relatively simple environment (i.c., an observation box).

In the present study, rats were exposed to the same behavioral habituation condition as in the previous study. As dependent variable the dentate granule cell response to perforant path stimulation was chosen. Interestingly, under these circumstances similar changes were found in this measure as in the visual cortical response. Thus, it seems that the perforant path - dentate granule cell response sensitively reflects alterations in tonic arousal level. It may be promising, therefore, to use this measure in attempts to further elucidate the neuromodulatory influence of PS deprivation.

References

Dement, W.C., Am.J.Psychiat., 1965, 122: 404-408.
Van Hulzen, Z.J.M. and Coenen, A.M.L. Physiol. Behav., 1984, 32: 557-563.
Steriade, M. Intern. Rev. Neurobiol. 1970, 12: 87-144.

LONG-TERM POTENTIATION AND THE PHOSPHORYLATION OF A PUTATIVE COATED VESICLE PROTEIN.

P.N.E. de Graan, L.H. Schrama, J. van Binsbergen, F.H. Lopes da Silva and W.H. Gispen

Epileptogenesis can be evoked in many brain areas by repetitive stimulation (kindling) with a short burst of high frequency electrical pulses (a tetanus). In the in vitro rat hippocampal slice preparation a single tetanus (50 pulses/s for 2 s) induces long-term potentiation (LTP), an increase in synaptic plasticity which lasts for hours. Concomitant with the LTP an increase in the phosphorylation of a 52 kDa protein can be detected in a crude synaptosomal fraction prepared from the tetanized slices. There appears to be a strong correlation between the degree of 52 kDa phosphorylation and the degree of LTP (as measured by the amplitude of the population spike). A semi-logarithmic plot of the percentual change in 52 kDa phosphorylation versus the change in postsynaptic potential per individual slice fits a straight line with a correlation coefficient of 0.71 ($p < 0.005$). The effect of tetanization on 52 kDa phosphorylation can also be found in synaptosomal plasma membranes (SPM) after subcellular fractionation of the crude synaptosomal fraction. The in vitro phosphorylation of the 52 kDa protein in SPM is Ca^{2+}/calmodulin and cAMP independent.

Purified bovine brain coated vesicles contain a 50 kDa phosphoprotein, which resembles our 52 kDa protein with respect to its insensitivity to Ca^{2+}/calmodulin and cAMP (Pauloin et al., 1982). Coated vesicles (CV) were isolated from rat brain and phosphorylated; the major phosphoprotein in CV comigrated with our 52 kDa protein from SPM. Indeed, the 52 kDa protein from SPM and CV's appear to be identical on basis of the following criteria: (i) isoelectric point; (ii) peptide mapping, and (iii) phosphoamino acid analysis.

CV's are involved in receptor-mediated endocytosis and presynaptic membrane recycling. Assuming the 52 kDa protein in SPM and CV to be identical, this would link the mechanism of LTP (and possibly of epileptogenesis) to presynaptic coated vesicle function. Modulation of 52 kDa phosphorylation in reponse to tetanic stimulation may thus be related to an increase in presynaptic membrane renewal and increased neurotransmitter release.

Reference

Pauloin et al., Nature 298, 1982: 574

NEURONAL PROPERTIES IN HIPPOCAMPAL SLICES FROM KINDLED RATS.

R.A. Voskuyl, H. Albus

Kindling is a model for epilepsy in which electroencephalographic and motor seizures are induced by repeated electrical stimulation of specific brain structures. An important aspect of the kindling model is that the effects are long lasting, if not permanent. If these long-lasting effects are based on altered neuronal properties, it may be expected that these properties will be preserved in brain slices. This would provide the opportunity to investigate the kindling model with the extensive electrophysiological testing methods that are possible with the in vitro technique.

The dentate area of the hippocampus, which receives input fibres from the entorhinal cortex coming in along the perforant path, was chosen as the target for kindling. Stimulating electrodes were placed in the perforant path both for kindling and evoking monosynaptic responses in the dentate area.

Recording electrodes were placed in the granule cell layer of the dentate area. Afterdischarges after kindling were recorded in the dentate area before behavioral manifestations were observed. During seizures the ECoG of the dentate area showed a highly characteristic pattern. A well known phenomenon in this pathway is long term potentiation (LTP) of the monosynaptic response after tetanic stimulation. During kindling long term potentiation was observed in some animals, but it was not essential for development of seizures. Inhibition of the first population spike, as evidenced by paired pulse depression, was enhanced. Although kindling in this pathway proceeded slower than in the amygdala, eventually all animals responded with full seizures to kindling stimulation.

Animals that had experienced full seizures at least 3 times were sacrificed and transverse hippocampal slices were prepared for electrophysiological investigation. We found no differences between slices from the kindled and unkindled side. Evoked responses appeared normal in all regions of the slice. No spontaneous epileptiform activity was observed, neither could it be provoked by repetitive stimulation or increasing K^+ from 3 to 8 mM. However, in paired pulse experiments in fascia dentata there was, except for the first 40-60 ms, a strong inhibition lasting more than 500 ms. In contrast, in CA1 cells only facilitation was seen. In slices from control animals inhibition in fascia dentata was less strong. These results agree with other reports indicating enhancement of inhibitory mechanisms, at least during

interictal periods. Possibly, this enhanced inhibition is a reaction to kindling to obtain seizures.

SINGLE ELECTRODE CURRENT AND VOLTAGE CLAMP ANALYSIS OF EPILEPTIFORM ACTIVITY IN VITRO.

H. Albus, R.A. Voskuyl

Aminopyridines have for a long time been known to stimulate transmitter release at chemical synapses. When injected into the hippocampus of animals 4-aminopyridine (4-AP) is a potent convulsant and produces an "animal model" of limbic epilepsy. Previously we have reported that 4-AP produces spontaneous extracellular field bursts in hippocampal slices (Voskuyl and Albus, in press). These bursts occurred in the CA1, CA3 and dentate areas. Two types of spontaneous field potentials could be recognized, one occuring at a rate of approximately 1/s and the other one at 1/7 sec. Using isolated segments of CA3 and CA1 areas, it appeared that the fast activity is initiated exlusively in area CA3 and propagates via synaptic pathways to CA1 neurons, whereas the slow activity can be initiated in both areas.

For the elucidation of the origin of the spontaneous field potentials intracellular measurements were carried out. Experiments were done under conditions of either constant current or constant voltage (the latter with a single electrode voltage clamp system). Recordings were obtained from CA1 cells in rat hippocampal slices, maintained at 34°C, with micro-electrodes filled with 3 M KCL. Under current clamp conditions, stimulation of the Schaffer collateral pathway produced an EPSP/IPSP sequence and one action potential. Bath application of 100 μM 4-AP resulted in an increase of the EPSP and an associated burst of action potentials. Also, an increased afterhyperpolarization was observed. Spontaneous large depolarizations (10-20 mV) with a burst of action potentials occurred in synchrony with spontaneous extracellular field potentials. Intracellular spontaneous bursts, associated with the fast or slow form of activity, differed in the, intervals between action potentials and the strength of the afterhyperpolarization.

When neurons were voltage clamped at a holding potential of -70 mV, fast spontaneous activity was accompanied by an inward current followed by a less pronounced outward current. On the other hand, with the slow form of activity the inward current was small or even absent, whereas the outward current was more pronounced. The outward current associated with slow activity

decreased at more negative holding potentials. The apparent reversal potential was -100 mV, suggesting that the current is carried by K^+ ions. These results, showing currents at constant holding potentials during spontaneous activity, indicate that spontaneous activity is at least associated with synaptic currents. They also suggest that different synapses are involved with the two types of spontaneous activity.

Reference

R.A. Voskuyl & H. Albus, Spontaneous epileptiform discharges in hippocampal slices induced by 4-aminopyridine, Brain Research, in press.

ANTIEPILEPTIC DRUGS AND NEURONAL MEMBRANES.

A.M.J. van Dongen, M.G. van Erp, R.J. van den Berg

Although antiepileptic drugs are among the most used medicines, their mechanisms of action either are unknown or controversial. Understanding the precise way in which they exert their antiepileptic action could give insight in the primary cause of the disorder, help to develop new superior drugs and aid in finding the most effective treatment for the different types of epilepsy. Usually two main hypotheses are put forward to explain the antiepileptic action:
- interference with synaptic transmission (c.q. effects on chemically regulated ionchannel systems);
- direct membrane effects (c.q. effects on voltage dependent ionchannels).

Here we will deal only with the latter hypothesis and discuss the effect of two drugs, valproate and phenytoin, on the functioning of sodium and potassium channels.

For our investigations we use a standard preparation: the node of Ranvier of a single myelinated peripheral nerve fibre. Under conditions of constant voltage (voltage clamp), sodium and potassium currents are elicited by depolarizing voltage steps, using fast voltage clamp system (Rijnsburger et al., 1985). The currents can be pharmacologically separated and the effects of the drugs can therefore be studied on either of these channel systems.

Phenytoin was shown to reduce selectively the sodium current in concentrations of 8-40 uM, suggesting a blockage of the sodium channels. The amount of blockage appeared to be modulated by membrane potential: depolarizing pulses with a duration of a few

seconds enhanced the blockage while hyperpolarizing pulses relieved it (Van Dongen et al. 1984).

Valproate was shown to reduce both sodium and potassium currents in concentrations of 0.5 - 9 mM. This effect was also modulated by voltage. Under current clamp conditions action potentials were recorded, showing reduction of excitability: the threshold and the refractory period increased, while the amplitude of the action potential and its maximum rate of rise decreased. The ability to fire repetitively was impaired.

These findings underline the importance of direct membrane effects for understanding the mechanism of action of both phenytoin and valproate. Blockage of sodium channels has been shown to be a common property of all antiepileptic drugs investigated sofar. The unexpected finding of the reduction of potassium current by valproate may help to explain its special status among the antiepileptic drugs.

References

Dongen, A.M.J. van, Erp, M.G. van, Berg, R.J. van den, "Blockage of sodium channels by the anti-epileptic drug phenytoin", Experientia 1984, 40: 1296-1297.

Rijnsburger, W.H., Berg, R.J. van den, Dongen, A.M.J. van, and Versluijs, C.A.A. An extremely fast voltage clamp for Ranvier nodes, 1985, submitted to J. Physiol. (P-paper).

ISOQUINOLINES AND BETA-CARBOLINES: LOOKING FOR ENDOGENOUS EPILEPTOGENIC SUBSTANCES.

M.G.P. Feenstra, T.J.A.M. van der Velden, O.R. Hommes

Various normal brain constituents may produce epilepsy when they penetrate into the brain in relatively high concentrations. Some of these compounds have a physiological role as excitatory neurotransmitter and are well studied. The majority of these compounds is, however, less well known. Amongst these latter are several monoamine neurotransmitter condensation products, e.g. beta-carbolines and isoquinolines. These substances are thought to be endogenously formed from tryptamine and phenylethylamine derivatives, respectively. The most likely mechanism is the condensation with an aldehyde e.g. formaldehyde. It has been shown that 5.10-methylenetetrahydrofolate can spontaneously liberate formaldehyde. It has also been shown that various isoquinolines and beta-carbolines have a number of interesting activities in

the brain, a.o. the epileptogenic action. We have tried to study the relation between the epilepsy induced by folate derivatives and the synthesis of the monoamine condensation products.
We have developed methods of determination for the direct condensation products of formaldehyde and dopamine or secrotonine. These products, 6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline and 6-hydroxy-1,2,3,4-tetrahydrobetacarboline, respectively, have been reported to be the isoquinoline and beta-carboline that are the most rapidly formed in vitro. Combined with the high concentrations of dopamine and serotonine in some brain regions, the above compounds may be considered as model compounds in the study of endogenous synthesis of isoquinolines and beta-carbolines. In addition, both compounds had been reported to be present in rat brain tissue.

The methods comprised high-performance liquid chromatography with amperometric detection. The detection limits were 1-5 ng/g brain tissue. When all possible artifactual formation had been excluded, our model compounds were not detectable in mouse brain or rat brain regions. Various pharmacological treatments which increased either the concentration of folate or those of dopamine and serotonine were studied in rats.

Only intracerebral injections of dopamine produced measurable concentrations of the dopamine-derived isoquinoline.

Our results stress the importance of using analytical techniques with a low liability to artifactual formation of these condensation products and the importance of verification of results obtained with one analytical technique by using another technique. Secondly, our results make it unlikely that folate derivatives induce epilepsy after conversion into monoamine condensation products.

Finally, our results do not exclude that certain isoquinolines and/or beta-carbolines are present in brain tissue. However, the present results suggest that it is unlikely that these compounds are synthesized in the brain.

INTRACORTICAL INJECTIONS OF FOLIC ACID IN THE RAT CHARACTERIZATION OF THE EPILEPSY AND COMPARISON WITH KAINIC ACID.

M.G.P. Feenstra, M.L.F. Schoofs, O.R. Hommes

Various folic acid derivatives are indispensable for normal cell functioning. However, they can elicit epileptiform responses when they penetrate into the brain in relatively high concentrations. This has been shown experimentally after microinjections in various brain areas, as well as in the cerebral ventricles, of several animal species. Peripheral administration in animals with local blood-brain barrier lesions also induces epilepsy.

In addition to this it has recently been shown that local intracerebral injections of folic acid may produce distant neurotoxic effects. In this respect folic acid was suggested to be comparable to the epileptogenic and neurotoxic glutamate analogue kainic acid. Since then, the actions of folic and kainic acid have been studied in a variety of experimental setups, including microinjections into the amygdala and the nucleus caudatus, and the results suggested that the effects of the two compounds were generally rather different and that a common mechanism of action was unlikely.

We have compared the effects of injections of folic and kainic acid in the motor cortex of the rat, a brain region where folic acid produces visible epileptiform effects in lower doses than in any of the above mentioned brain areas. The rats had chronically implanted polyethylene canulae in the right motor cortex and were injected with 0.5 µL when they were freely moving. Some of the rats were also equipped with electrodes to record the electrocorticogram (ECoG).

Folic acid induced partial motor epilepsy in dosages of 2 nmol and higher. The first visible events were light myoclonic jerks of the left hindleg. Between 2 and 20 nmol the duration and the intensity of the jerks were increased, dose-dependently the jerks spread more to the other limbs and partial focal motor seizures appeared. The ECoG showed sometimes immediately, but always within a few minutes, spikes, which first increased in amplitude and which correlated well with the occurrence of myoclonic jerks. After a certain time, depending on the dosage, the frequency and amplitude of the spikes decreased, no more seizures were recorded, and the epilepsy stopped.

Kainic acid in dosages of 0.3 - 20 nmol showed remarkedly different effects. The lower dosages only produced ECoG seizures

without interictal spiking. The highest dosage did produce interictal spikes, although not immediately. However, only during the ECoG seizures were jerks visible.

Further differences were: 24 h after 20 nmol kainic acid but not folic acid, some rats showed jerks; histological examination of the tissue surrounding the canula showed massive edema followed by neuronal degeneration in rats killed 2 h - 14 days after 20 nmol kainic but not folic acid; 2-3 weeks after 20 nmol kainic but not folic acid the concentrations of calcium in ipsi- and contralateral motor cortex as well as the ipsi- and contralateral thalamus had strongly increased.

These results suggest that the actions of folic and kainic acid in the cerebral cortex are completely different. The effects of folic are characterized by rhythmical jerks/spikes, a good correlation between ECoG and motor events and an absence of local or distant neurotoxicity. Kainic acid lacks the interictal rhythm, shows a poor correlation between ECoG and motor events and produces local and distant neurotoxicity. Preliminary results with bicuculline, carbachol, glutamate and N-methyl-D-aspartate suggest that these differences may reflect the difference between an increase of direct excitatory actions (kainate, glutamate, aspartate, carbachol) and a decrease of inhibitory actions (bicuculline, folic acid).

GENERATION AND PROPAGATION OF EPILEPTIFORM ACTIVITY IN THE HIPPOCAMPAL SLICE PREPARATION: EXPERIMENTS AND MODELLING.

J. Holsheimer, J.H. Koolstra and F.H. Lopes da Silva

The scope of this study is to investigate the processes by which epileptiform activity is generated and propagated in the hippocampal CA1-field. Therefore we used spatio-temporal field potential analysis and simulation methods, which provide information on (1) the distribution of electrical events at the soma-dendritic membranes of CA1 pyramidal cells, and (2) the propagation of epileptiform events.

The epileptiform events in the CA1 field of guinea pig hippocampal slices were investigated by simultaneous recordings of field potentials, using an array of eight electrodes at spacings of 0.1 mm. Epileptiform activity was provoked by adding 4-aminopyridine to the bathing medium.

For the analysis of the distribution of activity along the soma-dendritic membranes of the pyramidal cells the electrode array

was placed in the slice preparation in parallel to the axes of these cells. From the field potential profiles recorded in this way the Current-Source-Density was calculated.

Using a computer model of the pyramidal cells, by which several normal and pathological membrane processes were generated at specific sites of the soma-dendritic membranes, we were able to simulate the recorded field potential distribution fairly well. Therefore we had to decrease the conductivity of the fast K-channels and to generate depolarizing after potentials and Ca-spikes at the apical dendrite and afterhyperpolarization near the soma of the model cells.

For the analysis of the propagation of epileptiform events in CA1 the electrode array was situated in the pyramidal layer. Time delays between an epileptiform event recorded at different electrodes were estimated using the cross-correlation function. Different velocities were found, ranging from 0.3-0.5 m/s and from 1-2 m/s. These are probably related to various propagation mechanisms, which predominate at different extracellular K-concentrations.

SURGICAL THERAPY IN PARTIAL EPILEPSY.

R.M.Chr. Debets, C.W.M. van Veelen and C.D. Binnie

Surgical therapy of epilepsy is considered an useful alternative when medical treatment has failed and seizures continue. More than 50 years ago Penfield carried out focus excision in temporal lobe epilepsy for the first time and the number of sucessful operations has increased due to a more effective presurgical evaluation.

As at least 20% of patients with partial epilepsy are poorly controlled, surgical therapy should be considered more often since data from the literature and our own data indicate that 60 - 80% of the patients show a complete or almost complete postoperative relief of seizures.

In general presurgical evaluation is considered when there is no diffuse brain disorder as reflected by an IQ under 70-75.

The criterium of "incapacitating" seizures shows a considerable interindividual variation and depends on the social status, aims and risks in daily life. A rather low seizure frequency can be incapacitating for an individual patient, whereas in others, with

more frequent attacks, there is no obvious influence on daily life especially when these occur during the night. Seizures should be resistent to medication and trials with major anti-epileptic drugs must have been carried out. Good results are sometimes obtained with high dose monotherapy. If results are not satisfactory, anti-epileptic medication is tapered to the lowest dose in relation to seizure frequency. For discussing the indication for surgical treatment in general and to decide whether or not presurgical intracranial EEG seizure recording in combination with video monitoring are necessary, patients are presented to the Werkgroep Neurofysiochirurgie in Utrecht (Chairman: Dr. J. van Manen, Academisch Medisch Centrum, Amsterdam) that gathers bimonthly.

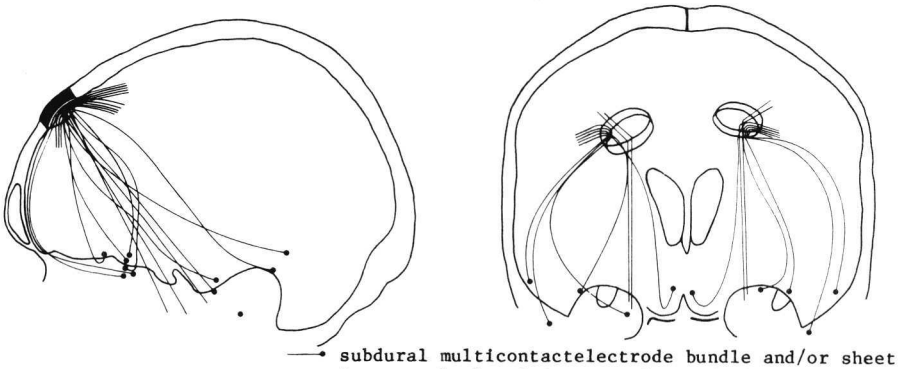
When a patient is considered a candidate for surgical therapy the investigations proceed with neuropsychological testing, seizure recording, nuclear magnetic resonance scanning and positron emission tomography.

Neuropsychological testing is necessary to determine lateralization of speech and for determination of a memory deficit in addition to general parameters as intelligence and personality. Careful evaluation of cognitive functioning is necessary and is performed before as well as after resection of an epileptic focus. Mild psychopathology is not considered a contra-indication for surgery, but cooperation of the patient is essential.
Psychiatric expertise can be necessary and postsurgical treatment is often advised in these cases.

Evaluation for operation depends upon seizure type as well as the presence of a circumscribed focal epileptiform EEG abnormality in the initial phase of a seizure. Seizure recordings with scalp electrodes can show a focal abnormality, but with intracranial EEG recordings in selected cases, however, a more effective focus detection is possible especially in frontal and mesial temporal structures of the brain.

In partial epilepsy epileptiform discharges often originate from these mediobasal structures. Recordings are continued until a sufficient number of representative seizures are obtained for analysis with a longterm EEG-video monitoring system.

In the Netherlands subdural electrodes are used in combination with only a limited number of depth electrodes in order to decrease the risk related to the use of depth electrodes (see figure).



With this approach there was no morbidity or mortality in 22 patients examined in recent years.

After these assessments there is a final discussion in the "Werkgroep" and with the patient. If surgery is indicated it can be performed under general and if necessary under local anaesthesia. Corticography during the operation is used for additional focus localization. Cortical mapping with electrostimulation under local anaesthesia is performed when vital functions are at risk.

The apporach for presurgical evaluation follows a stepwise procedure and lasts about 6 months. In general no somatic or psychic detoriation occur after surgery, except for a small visual field defect and slight changes in the results of neuropsychological tests. Neuropsychological tests can even show better results after surgery. There is a tendency that psychiatric disorders diminish too.

Medication is continued after surgery, but decreasing the dose and discontinuation of drugs, after 2 years, contributes to further improvement of cognitive functioning.

In conclusion, neurosurgical therapy of epilepsy deserves more attention according to the successrate and limited risk in selected patients.

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