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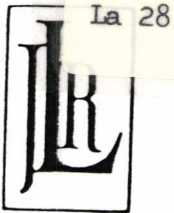

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8. THE EFFECT OF INHIBITORS OF THE CHOLESTEROL SYNTHESIS ON HMG-CoA REDUCTASE ACTIVITY IN THE HUMAN HEPATOMA CELL LINE HEP G2. L. H. Cohen, A. Boogaard, and H. J. Kempen. *TNO Gaubius Inst. for Cardiovascular Res., Herenstraat 5d, 2313 AD Leiden, The Netherlands.*

Blocking the pathway to cholesterol at specific sites causes accumulation of intermediates before and depletion of metabolites behind the blockade. Both effects may influence the reductase activity and hence may give insight in regulatory mechanisms. We already showed that in Hep G2 cells compactin, an inhibitor of the reductase itself, gave rise to an induction of the reductase activity (measured after removal of the drug), which was prevented partially by LDL and totally by mevalonate (Biochem. J. 222 (1984) 35-39). In the experiments to be described here, we used U18666A as inhibitor of 2,3-oxidosqualene cyclase, buthiobate and ketoconazole as blockers of the C14-demethylation of lanosterol and triparanol, an inhibitor of desmosterol reductase. None of these substances inhibited the reductase activity in Hep G2 cell homogenates. After an 18-20 h incubation of the cells with different concentrations of the drug, the HMGCoA reductase activity was determined and the inhibition of the cholesterol synthesis and accumulation of intermediates were determined by incorporation of [^{14}C]acetate or [^{14}C]-mevalonate into nonsaponifiable lipids, identified by TLC (in the control without drugs the ^{14}C-label was incorporated only into cholesterol). In some cases accumulation of intermediates was confirmed by gas chromatographic analysis. Inhibition of the cholesterol synthesis by U18666A resulted in a decrease of the reductase activity with a minimum at 0.3-0.5 μM U18666A; however at concentrations higher than 3 μM a marked and concentration-dependent increase of the reductase activity was found. The accumulated ^{14}C-labeled intermediate formed at the lower concentration of U18666A was different from those formed at the high concentrations of the drug. The latter were probably 2,3-oxidosqualene and squalene-2,3:22,23-dioxide, whereas at the low U18666A concentration one component was found, which behaved as a polar sterol in TLC. This finding suggests that this compound may be involved in the lowering of the reductase activity. The U18666A-induced increase was additive to the activity enhancement of compactin and was not abolished by mevalonate. In the presence of 30 μM U18666A (^{14}C-acetate incorporation into cholesterol was $< 3\%$ of control) the increase due to compactin could be prevented by addition of mevalonate. This indicates the existence of a nonsterol effector in addition to a sterol dependent regulation. Surprisingly LDL, which lowered the reductase activity itself, enhanced the effect of U18666A at concentrations higher than 3 μM. Incubation of the cells with buthiobate or ketoconazole resulted in a concentration-dependent decrease of the reductase activity accompanied with a lanosterol accumulation. No other nonsaponifiable lipid was detected. With triparanol we also found a concentration-dependent lowering of the reductase activity, now accompanied with an increase of the desmosterol content of the cells. In this case as well as in the previous described one it is possible that from the accumulated sterols metabolites are formed which suppress the reductase activity. Another explanation may be that the sterol accumulating in the membranes directly influences the reductase activity.

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