recent data indicating a role of this substance in regulating cellular functions, often opposing 3′5′AMP[10].

Methods and results. Wistar male rats, fasted for 12 h, of the approximated weight of 200 g were used.

Edema was produced in the left prosterior paw, following Winter method, by a subplantar injection of 100 ng/rat of PGE_2 and $PGF_2\alpha$ dissolved in 0.1 ml of physiological solution. Fluid paw volume was measured with a pletismograph before the injection and 30, 60 and 120 min after.

The paw's volume increase after subplantar injection of 0.1 ml physiological solution was assumed as a control value. 10 mg/kg total weight of 3′5′AMP and 3′5′GMP was injected i.p. 15 min before subplantar injection of PG. The dose was chosen for its scarse or no effects on cardiovascular function, which had been confirmed beforehand. The production of edema in rats paw, following a 3′5′AMP or 3′5′GMP injection alone, was also controlled at the dose of 1 mg in 0.1 cm^3 by subplantar injection.

Prostaglandins were kindly supplied by Dr. PIKE (Upjohn Kalamzoo, USA) and 3′5′AMP and 3′5′GMP by Dr. TOFANETTI (Boehringer, Mannheim, BRD). Results are listed in Tables I and II.

Discussion. Inhibition of histamine release and enzymatic lysosomal extrusion[11], as well as macrophagic migration, and leucocyte chemiotactic reaction is known to be determined by an increase of intracellular 3′5′AMP concentration, following a stimulation of adenylcyclase or an inhibition of phosphodiesterases[4,12]. On the contrary, an increase of intracellular 3′5′GMP concentration results in opposite effects[10].

The present observations on rat's paw edema from PG confirm the opposite effect of these cyclic nucleotides. In effect prostaglandins edema development is inhibited by 3′5′AMP, whereas 3′5′GMP does not interfere with, or increase, the edema responsiveness to PG administration. The inhibiting effect of 3′5′AMP is more evident in PGF induced edema, the time course of which was not modified by 3′5′GMP. Also PGE-induced edema is inhibited by 3′5′AMP, whereas it is slightly increased by 3′5′GMP pretreatment.

The respective behaviour of the cyclic nucleotides also shows some differences in their primary edema-induced activity, since 3′5′AMP shows no effect with regard to this (and behaving thus as a physiological solution), whereas a late, weak edema is induced by 3′5′GMP administration. The fact that a different behaviour is shown by cyclic nucleotides by themselves, or when tested on a PGE or PGF induced edema, suggests that a different mechanism of action underlies this difference.

This is the case of rat liver: the release of enzymes from lysosomes fraction is inhibited by β-stimulating drugs or 3′5′AMP administration and stimulated by 3′5′GMP[13], and the behaviour of protease release by human leukocytes[14] and of smooth muscle[15] contraction mechanisms is similar.

Other experiments have also shown that 3′5′AMP pretreatment, or β_2-stimulating drugs administration (which are well known stimulators of adenylate cyclase activity) have an inhibiting effect on $PGF_2\alpha$-induced bronchospasm. 3′5′GMP pretreatment does not modify the $PGF_2\alpha$-induced bronchospasm and counteracts the manifestation of the bronchodilator effect due to PGE_2[16].

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Diphasic Action of the Aliphatic Amide, HOE 17879, on Hepatic Microsomal Drug Metabolizing Enzymes in the Mouse

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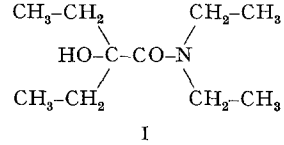
Summary. α-Hydroxy-α-ethylbutyryl-diethylamide (HOE 17879) influences the hepatic microsomal drug hydroxylating enzyme system of mice in a two-phasic way: First it reduces the overall reaction of drug hydroxylation but not the single enzymes whilst in the second period it increases enzyme activities.

A great number of substances exert a diphasic action upon the hepatic microsomal drug-hydroxylating enzyme systems by inhibiting them in the first period of action, and increasing their activities in the second period of action due to induction of their biosynthesis (see [2–9], for instance). This paper describes the diphasic action on liver microsomal drug-metabolizing enzymes of the aliphatic amide, HOE 17879 (α-hydroxy-α-ethylbutyryl diethylamide, I), a substance which possesses little pharmacological activity by itself[10] but influences, in a diphasic manner, the narcosis times of a series of narcotics capable of being metabolized in the liver[11]. Because of its relatively simple structure, this substance might well be a tool for studying these enzyme inhibition effects which lead afterwards to enzyme biosynthesis induction. By the authors cited above, only the overall reaction of drug hydroxylation has been studied. For us, it seemed to be interesting to investigate the behaviour of the single components of the microsomal respiratory chain,



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too, e.g. of NADPH dehydrogenase, NADPH: cytochrome c oxidoreductase ('cytochrome c reductase'), NADPH: neotetrazolium chloride oxidoreductase ('neotetrazolium reductase'), and cytochrome P-450, in order

to get insight into the mechanism of enzyme induction by xenobiotics. We included hexobarbital sleeping-time measurements into our study because hexobarbital sleeping-time is widely concerned as an in-vivo indicator of hepatic drug-metabolizing enzyme activity insofar as prolonged sleeping-time reflects enzyme inhibition, and shortened sleeping-time, enzyme activation (by biosynthesis induction[12–17]) although one cannot exclude the possibility that HOE 17879, as well as all other substances acting on hexobarbital sleeping-time[4,6], may change other factors such as brain susceptibility and body distribution of hexobarbital, too. Besides this, in separate experiments with mice, we measured D-tubocurarine, and guaiacol glycerol ether (guaifenesin) induced muscle relaxation times, in order to get two other in-vivo parameters for drug metabolism as influenced by HOE 17879. Parts of our findings have already been reported in a previous paper[18].

Experimental. The experimental animals, male mice (of the IMRI strain), were distributed in 3 groups consisting of 20 animals, each, and had free access to food and drinking water. The animals of group I served as a control, those of groups II and III received the substance HOE 17879, dissolved in polyethylene glycol, in a single i.p. dose of 150 mg/kg body weight 40 min and 24 h before sacrifice; the control animals were injected the same amount of the solvent. At the time of sacrifice, 10 mice of either group were injected 100 mg/kg body weight sodium hexobarbital for measurement of the hexobarbital sleeping time; all other mice of the groups were killed by decapitation. The livers were removed and frozen quickly by immerging them in liquid nitrogen. They were stored at $-20\,^{\circ}C$ until they were handled further.

After thawing, 5 livers of each group were homogenized separately in ice-cold isotonic potassium chloride solution

[1] Acknowledgments. The authors are indebted to their colleague, Dr. K. SCHMITT, for making the substance HOE 17879 available. They wish to thank to Mr. MAYER, Mr. PAULUSCH, Mrs. FEY, Mrs. STEIN, and Miss FEHLINGS for their skilful assistance in the project.

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Table I. Liver enzyme changes under HOE 17879 given 40 min resp. 24 h before

	Control	40 min before	24 h before
$NADPH_2$ dehydrogenase (units/g liver)	1.22 ± 0.32 ($100\% \pm 26\%$)	1.17 ± 0.14 ($96\% \pm 11\%$) n.s.	1.39 ± 0.20 ($114\% \pm 16\%$) n.s.
Neotetrazolium reductase (units/g liver)	0.302 ± 0.037 ($100\% \pm 12\%$)	0.352 ± 0.025 ($117\% \pm 8\%$) $p < 0.05$	0.410 ± 0.033 ($136\% \pm 11\%$) $p < 0.01$
Cytochrome c reductase (units/g liver)	29.0 ± 6.3 ($100\% \pm 22\%$)	30.9 ± 1.8 ($106\% \pm 6\%$) n.s.	44.0 ± 2.7 ($152\% \pm 9\%$) $p < 0.01$
Cytochrome P-450 (Δ E/g liver)	0.061 ± 0.010 ($100\% \pm 16\%$)	0.051 ± 0.030 ($84\% \pm 49\%$) n.s.	0.70 ± 0.010 ($149\% \pm 21\%$) $p < 0.01$
N-Demethylase (units/g liver)	0.202 ± 0.057 ($100\% \pm 28\%$)	0.136 ± 0.021 ($67\% \pm 10\%$) $p < 0.01$	0.357 ± 0.061 ($177\% \pm 30\%$) $p < 0.01$
Nitroanisol-*O*-demethylase (units/g liver)	0.140 ± 0.039 ($100\% \pm 28\%$)	0.0786 ± 0.0169 ($56\% \pm 12\%$) $p < 0.01$	0.1904 ± 0.045 $136\% \pm 32\%$) $p < 0.05$
Anisic ester-*O*-demethylase (units/g liver)	0.092 ± 0.0446 ($100\% \pm 49\%$)	0.0424 ± 0.0071 ($47\% \pm 8\%$) $p < 0.05$	0.180 ± 0.034 ($200\% \pm 38\%$) $p < 0.01$
Biphenyl 4-hydroxylase (units/g liver)	2.28 ± 0.23 ($100\% \pm 10\%$)	1.60 ± 0.31 ($70\% \pm 14\%$) $p < 0.05$	2.75 ± 0.23 ($121\% \pm 10\%$) $p < 0.05$

The numbers in brackets denote percentage of control.

Table II. Drug action time changes under HOE 17879 given 40 min resp. 24 h before drug administration

	Control	40 min before	24 h before
Hexobarbital sleeping time (min)	24.4 ± 5.3 (100% ± 22%)	67.3 ± 17.1 (280% ± 7%) $p < 0.001$	7.2 ± 2.5 (29% ± 10%) $p < 0.001$
D-Tubocurarine relaxation time (min)	3.02 ± 1.20 (100% ± 39.7%)	7.71 ± 3.37 (255.3% ± 111.6%) $p < 0.01$	0.62 ± 0.49 (20.5% ± 16.2%) $p < 0.001$
Guaiacol glycerol ether relaxation time (min)	38.5 ± 13.7 (100% ± 35.6%)	86.9 ± 13.9 (225.7% ± 36.1%) $p < 0.001$	22.7 ± 3.5 (59.0% ± 9.1%) $p < 0.01$

The numbers in brackets denote percentage of control.

with a teflon homogenizer of the Potter-Elvehjem type[19]. From these homogenates, 12,000 × *g* supernatants and microsomes were prepared by centrifugation. Besides this, from the remaining 5 livers of all groups, microsome specimens were prepared by the calcium chloride precipitation method[20, 21].

In the calcium-precipitated microsomes, the activities of the following enzymes were assayed: NADPH dehydrogenase, neotetrazolium reductase following DALLNER et al.[22] and LESTER and SMITH[23], *p*-nitroanisol *O*-demethylase by a modification of the procedure of NETTER and SEIDEL[24], and biphenyl 4-hydroxylase following CREAVEN et al.[25] as modified by BRIDGES (personal communication). In the microsomes prepared by ultracentrifugation, the cytochrome P-450 content was measured following OMURA and SATO[26]. In the 12,000 × *g* supernatants, the activities of the following enzymes were determined: cytochrome c reductase following CLEVELAND and SMUCKLER[27], aminopyrine N-demethylase following LEBER et al.[28] and NASH[29], and anisic ester *O*-demethylase by the method of BEYHL et al. (1976, in preparation). For the determinations of muscle-relaxation times, the mice which were treated with HOE 17879 in the same way as in the first experiment, were injected i.v. D-tubocurarine (curarin Asta) in a dose of 0.1 mg/kg, and guaiacol glycerol ether (MY 301 forte) in a dose of 500 mg/kg, resp.

Results and discussion. The results are listed in the Tables. 40 min after the application of HOE 17879, biphenyl hydroxylase, and all three demethylase activities are diminished. This means that drug metabolism is inhibited by this substance in term of the overall reaction. But the activities of the single components of the microsomal respiratory chain are unchanged at that time: NADPH dehydrogenase, cytochrome c reductase, and cytochrome P-450 remain constant, neotetrazolium reductase activity being even slightly increased. These results, which at first look seem to conflict with each other insofar as the drug hydroxylation (and desalkylation) is the result of the cooperation of the single components of the respiratory chain, can be explained as follows: The molecules of the substance HOE 17879 combine in a drug-like manner with the drug-binding sites of the microsomal end oxidase, at the cytochrome P-450 level, and by this, stop the drug-binding and monooxygenation; but they do not influence either NADPH dehydrogenation or cytochrome c reductase. The increase of neotetrazolium reductase activity at this time may be due to an accumulation of reduction equivalents coming from NADPH since the 'normal' electron flow (whatever this means) to cytochrome P-450 and oxygen is stopped.

At the 24 h interval, both the overall reactions of drug hydroxylation, and the single components are increased: All enzyme activities (except NADPH dehydrogenase) and the cytochrome P-450 content are higher than the control levels. The only enzyme which seems not to be induced by HOE 17879 is NADPH dehydrogenase, since in microsomes NADPH is oxidized by other reactions besides drug metabolism, and therefore no significant increase of NADPH oxidation can be detected. We should like to suggest that, as a consequence of this drug hydroxylation impairment, biosynthesis of the constituents of the microsomal respiratory chain is induced.

The behaviour of the in-vivo parameters of drug metabolism, namely hexobarbital sleeping-time and D-tubocurarine, and guaiacol glycerol ether relaxation times, run parallel with each other: In the first period of action of HOE 17879, the drug actions are prolonged, and in the second period, we notice an abbreviation of the actions of the drugs as compared to control animals. Hexobarbital blood levels in animals treated with HOE 17879 40 min before, decrease more slowly than those in control animals, up to 150 min after application[11], which shows that hexobarbital metabolism is impaired by HOE 17879 in its first period of action.

The termination of the muscle-relaxing action of D-tubocurarine can be ascribed to metabolic degradation of the drug in the liver, as suggested by BUZELLO et al.[30],

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and the same has been shown by GIRI[31] for guaiacol glycerol ether. For this substance it could be shown that it is metabolized in the liver by *O*-demethylation[31, 32] and ring hydroxylation (v. KEREKJARTO and BEYHL 1976, in preparation) in its first step of metabolism.

It is unlikely that the parallel behaviour of hexobarbital sleeping-time and tubocurarine and guaiacol glycerol ether muscle-relaxation times is to be ascribed to parallel susceptibility changes of the target organs to these three drugs caused by HOE 17879 – although one cannot rule out this possibility definitively. It seems more likely that this parallelism can be ascribed to parallel and common changes in the metabolism of these drugs brought about by HOE 17879 in the liver microsomal drug hydroxylating enzyme system, namely metabolism inhibition in the first action period, and metabolism stimulation in the second. Thus, the in-vivo measurements of drug metabolism agree well with the in-vitro measurements of hepatic drug metabolizing mixed-function oxidase activities.

Structure-Activity Relationships of Cyclic Acetylcholine Analogues of the Piperidinol and Thiacyclohexanol Series

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Summary. The hydrochlorides and methiodides of 1-methyl-3- and 4-acetoxypiperidine and their sulphonium analogues are cholinergic agonists. They are substrates for acetylcholinesterase. The sulphonium compounds have a 78(-524)-fold higher activity than its nitrogen analogues.

In the course of our investigations of the structure-activity relationships of cyclic acetylcholine analogues, we studied the action of the hydrochlorides and methiodides of 1-methyl-3-acetoxypiperidine and 1-methyl-4-acetoxypiperidine on muscarinic receptors[2]. These compounds (I, II, IV and V) may exist in a number of chair and boat forms, in which the methyl groups on nitrogen and the ester side-chain may be orientated axially or equatorially (Figures 1 and 2).

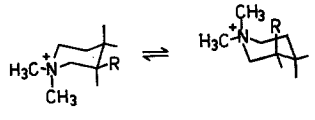

Fig. 1. Conformation equilibrium of compound V. Compound II has the acetoxy-group at C–4. R = $-O-\overset{\overset{O}{\|}}{C}-CH_3$ (chair forms only are shown.)

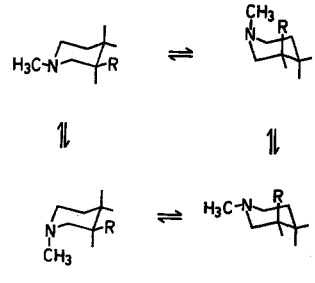

Fig. 2. Conformation equilibria of compound IV. Compound I has the acetoxy-group at C–4. R = $-O-\overset{\overset{O}{\|}}{C}-CH_3$ (chair forms only are shown.)

Population analyses of the substituted piperidines I and IV and their quaternary ammonium salts II and V in solution have been carried out by ^{1}H-NMR spectroscopy[3, 4]. With the ammonium ions II and V, analyses assuming an equilibrating system between the 2 chair conformers shown in Figure 1 have been carried out. The possibility of the presence of a measurable part of boat forms has been ruled out, as steric effects (or van der Waals interactions) are too large to permit stabilization of boat forms of the substituted ammonium ions. In the case of the tertiary amines I and IV, however, 4 conformers should be taken into account because of the presence of nitrogen pyramidal inversion, even if the stable conformations are chair forms. The population analyses in solution show that the methyl group on nitrogen of compounds I and IV is orientated equatorially (as a result of a high pyramidal inversion rate and relatively large free energies for the methyl group on nitrogen in the axial conformation). The populations of the conformers of compounds I and II with the equatorially or axially ester side-chain are 31:69% respectively 57:43%, the values for compounds IV and V are 34:66% respectively 58:42%.

As a result of the nitrogen pyramidal inversion and of the chair/chair-inversion of the piperidine ring system, the individual conformers shown in Figures 1 and 2 cannot be isolated. Therefore, the question remains: What is the actual active conformation of the piperidine derivatives at the muscarinic receptor? It is not necessarily any correlation between the energetically preferred conformation in solution and the active conformation at the receptor. Preliminary pharmacological investigations with compound IV and 3-acetoxyquinuclidine show that a boat form appears to be responsible for the reaction of IV with the muscarinic receptor in which the methyl group on nitrogen is axial[5] (Figure 3).

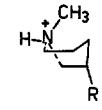

Fig. 3. The active conformation of 1-methyl-3-acetoxypiperidine at the muscarinic receptor[5]. R = $-O-\overset{\overset{O}{\|}}{C}-CH_3$.

[1] We thank the German Research Association for support of this work, and Miss CHRISTIANE ROTH for her valuable assistance in carrying out the investigations.

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