spite of a 10 times higher partition coefficient. Graphical analysis of the kinetic data showed a mixed type of inhibition for all the compounds investigated.

For statistical analysis of the data, the linear regression line was calculated by the method of least squares, from the logarithmic values of the partition coefficients (log P) of the drugs and from their respective inhibitory potency (log $1/K_i$) on the serum cholinesterase. The regression equation was $\log 1/K_i = 0.450 \log P + 4.200$ $n = 11$; $r = 0.922$.

Linearity of the regression function was tested by the F-distribution.

Discussion. Our investigations have shown a statistically significant correlation coefficient ($p < 0.001$) between the 2 parameters (log P and log $1/K_i$) investigated. This suggests that hydrophobicity of the β-sympatholytics plays a significant part in the inhibitory potency of the drugs on the serum cholinesterase. The low slope of the regression line indicates, however, that the mechanism of action must be more complex, as was pointed out by HANSCH and DUNN[6] for a variety of biological systems. The unexpected low inhibitory potency of Kö 1124 can probably be explained by a steric effect of the m-isobutyl substituent of this compound. This effect, together with the experimental difficulties in estimating the partition coefficients of extremely polar compounds such as Kö 1439, may be responsible for a statistically significant deviation of the regression line from linearity ($F = 6.03$; $p < 0.01$). Omitting these 2 compounds (Kö 1124 and Kö 1439) from calculation the following regression line was obtained:

$$\log \frac{1}{K_i} = 0.414 \log P + 4.300;\ n = 9;\ r = 0.984.$$

As the test quotient for this regression function ($F = 0.66$) did not exceed the significance limit ($p > 0.05$), it can be assumed that this regression function is linear.

The relationship between the hydrophobic properties of the β-sympatholytics investigated and the inhibitory potency on the serum cholinesterase might be interpreted as a fairly non-specific binding of drug to the enzyme protein, because the mixed type of inhibition suggests that not only the free enzyme but also the drug enzyme complex is affected by the drugs. Stereospecifity, which is typical for the specific antiadrenergic activity of this group of drugs seems not to be involved. In our experiments the K_i value of (−)–Kö 1313 was essentially the same as that of (+)–Kö 1313.

Zusammenfassung. Die hemmende Wirkung von β-Sympatholytica auf die Serum-Cholinesterase wird als unspezifische Wirkung angesehen und kann mit ihren hydrophoben Eigenschaften korreliert werden.

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Changes in Brain Acetylcholinesterase Activity of Young Rats after Chronic Treatment with Tremorine

Oxotremorine or tremorine-induced tremors may offer some information concerning possible mechanisms involved in tremor diseases in general and in Parkinsonism in particular. Acute administration of tremorine[1,2] or its active metabolite, oxotremorine[3,4] causes an increase of brain acetylcholine and a causal relation between increased acetylcholine and the associated produced tremor by these agents has been proposed[2]. The present study was designed to investigate biochemical changes in rats treated chronically with tremorine, beginning at 60 days of age.

Methods. Sprague-Dawley male rats were purchased from Simonsen Laboratories, California and delivered to our laboratory at 60 days of age. 4 animals received daily for 7 days, the vehicle 0.9% NaCl i.p.; 4 animals received daily for 7 days either a) tremorine, 7.5 mg/kg body weight, or b) tremorine, 22.5 mg/kg body weight, or c) tremorine 30 mg/kg body weight. Body weights were recorded throughout the experimental period. 4 days following the 7-day injection period all animals were sacrificed by decapitation, the brains were rapidly removed, blotted free of moisture and weighed. The following CNS areas were rapidly dissected and used for biochemical determinations: cerebral cortex (gray matter only), caudate, hypothalamus and cerebellum.

Acetylcholinesterase (AChE) was determined colorimetrically by means of a Beckman DU spectrophotometer, using the rate of hydrolysis of the substrates acetylthiocholine (AcTCh), according to the method of ELLMAN et al.[5]. The determination of the enzyme activity was carried out 37 °C. Homogenate consisted of 1 mg tissue per ml of 0.07 M phosphate buffer, pH 8.0, prepared with 0.07 Na_2HPO_4 and 0.07 KH_2PO_4. The final reaction mixture for determining AChE activity consisted of 2.9 ml pH 8.0 buffer, 0.1 ml homogenate, 100 µl (0.001 M) dithiobisnitrobenzoic acid (DTNB), and 20 µl acetylthiocholine iodide (0.075 M). Enzyme activity was expressed as µmoles of substrate hydrolyzed per min per g of wet tissue. To determine whether the means of parameters measured in controls and tremorine-treated rats differed significantly, the t-test for nonpaired data was applied[6].

Results. The 7-day treatment schedule was chosen in an attempt to produce spontaneously occuring tremors after chronic administration of tremorine. However, because

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the experimental animals were gaining very little weight after the 4th injection of tremorine, drug treatment was stopped and a 4-day partial recovery was allowed. The experimental animals began gaining weight within 2–3 days after cessation of the drug treatment. No spontaneous tremors were observed either during or after the drug treatment period.

The tremorine-treated animals exhibited various patterns of convulsive movements (Table I). The onset of these symptoms ranged from 6–18 min in the low-dose tremorine-treated group to 6–12 min in the other 2 treated groups. The onset or pattern of convulsive movements did not change significantly during the 7-day treatment period.

The group of animals which had received the lowest dose of tremorine (7.5 mg/kg body weight) exhibited only light jaw tremor whereas the other two groups exhibited more intense patterns of convulsive movements including forelimb clonus.

During the 11-day experimental period control animals gained an average of 45 g; the group of animals treated with tremorine, 7.5 mg/kg body weight, gained 23 g, those treated with 22.5 mg gained 26 g and those treated with 30 mg gained 22 g (Table II). No differences were observed in the brain weights between the control and the drug-treated groups.

Total DNA, RNA and protein contents were investigated in the 4 CNS areas but no differences were observed between controls and tremorine-treated rats. The results, therefore, are not presented here. Acetylcholinesterase activity was markedly higher in the cerebral cortex of the animals which received 30 mg/kg weight of tremorine as compared to controls (Table III). No differences were observed among the remaining CNS structures studied. All treated animals exhibited automatic symptoms which included salivation, piloerection, sereve lacremation and in the high-dose treated animals, eyebleeding and diarrhea during the daily treatment[7].

Discussion. Chronic administration of tremorine markedly influenced the rate of growth of rats with the results primarily attributed to lack of eating. Whereas

Table I. Convulsive-like movements in young rats treated chronically with tremorine

Treatment[a]	Onset of convulsive movements (min)	Type of convulsive movements[c]
Tremorine 7.5 mg/kg body wt.	6–18[b]	Light jaw and vibrissae tremor
Tremorine 22.5 mg/kg body wt.	6–12	Head and body tremor Whole body jerking Opisthotonus Forelimb clonus Groaning
Tremorine 30 mg/kg body wt.	6–12	Head and body tremor Whole body jerking Opisthotonus Forelimb clonus Groaning

[a] Animals were treated daily for 7 days; similarly, controls received NaCl. [b] Range of onset during the 7-day experimental period. [c] See Results for details.

Table III. Acetylcholinesterase activity in several CNS structures in rats treated chronically with tremorine

Treatment[a]	CNS structure	Acetylcholinesterase activity (μmoles AcTCh hydrolysed/min/g wet tissue)
Control	Cerebral cortex	4.75 ± 0.36[b]
	Cerebellum	3.35 ± 0.09
	Caudate	31.49 ± 1.56
	Hypothalamus	7.26 ± 0.37
Tremorine 7.5 mg/kg	Cerebral cortex	5.29 ± 0.52
	Cerebellum	3.32 ± 0.11
	Caudate	28.43 ± 1.44
	Hypothalamus	7.57 ± 0.50
Tremorine 22.5 mg/kg	Cerebral cortex	4.27 ± 0.20
	Cerebellum	3.43 ± 0.20
	Caudate	33.28 ± 1.00
	Hypothalamus	6.71 ± 0.31
Tremorine 30 mg/kg	Cerebral cortex	7.20 ± 0.32 (< 0.001)[c]
	Cerebellum	3.57 ± 0.07
	Caudate	28.90 ± 1.83
	Hypothalamus	7.17 ± 0.20

[a] Animals were treated daily for 7 days before sacrifice; similarly, controls received NaCl; [b] Mean ± SE; [c] Numbers in parentheses are P values for comparison to controls.

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Table II. Changes in body weight during tremorine treatment

Treatment	Days from beginning of treatment										
	1	2	3	4	5	6	7	8	9	10	11
Control	301 ± 3[a]	310 ± 2	315 ± 2	316 ± 3	319 ± 3	325 ± 3	324 ± 3	336 ± 2	338 ± 3	336 ± 4	346 ± 6
Tremorine 7.5 mg/kg	269 ± 4	277 ± 4	276 ± 3	275 ± 3	277 ± 1	278 ± 1	277 ± 2	281 ± 2	283 ± 2	281 ± 3	292 ± 4
Tremorine 22.5 mg/kg	289 ± 7	296 ± 7	299 ± 8	305 ± 4	303 ± 7	306 ± 6	290 ± 9	299 ± 9	299 ± 9	291 ± 11	315 ± 9
Tremorine 30 mg/kg	278 ± 4	282 ± 4	282 ± 5	286 ± 5	286 ± 6	290 ± 5	287 ± 5	286 ± 4	292 ± 6	294 ± 7	300 ± 9

[a] Mean ± S.E.

control animals gained 45 g during the 11-day experimental period, tremorine-treated animals gained an average of 24 g. This effect on body weight was not reflected on brain weight. When brain weights were directly compared among the groups, they were not significantly different.

The lack of eating in the treated animals would be interpreted to reflect possible effects of tremorine on the hypothalamus although no direct supportive evidence is known. Another factor besides lack of eating which might be involved in the loss of weight could be the effect of the daily produced tremor, a stressful stimulus, on the adrenocortical system. An increased secretion of adrenocortical steroids due to stress would result in both catabolism and antianabolism of muscle protein which adrenocortical steroids are known to exert.

Several studies[2-4] have attempted to explain the tremor phenomena produced by tremorine and oxotremorine by increased levels of brain ACh. Moreover, attempts have been made to explain increased levels of ACh, either by decrease in activity of AChE, the hydrolysing enzyme of ACh, or increase in the activity of choline acetyltransferase, the synthesizing enzyme of ACh. The present marked increase of AChE in the cerebral cortex observed with the highest dose of tremorine (Table I) does not substantiate such a correlation. Also, recent studies by BARTOLINI et al.[3] have shown that ACh level is raised in the diencephalon-midbrain but not in the cerebral cortex after acute oxotremorine administration. Since in the present study no changes were observed in the AChE activity in the diencephalon-midbrain, the increased ACh levels observed by BARTOLINI et al.[3] cannot be explained by changes in the activity of the hydrolysing enzyme. It is suggested that the high AChE activity in the cerebral cortex after tremorine observed in this study may reflect a high turnover rate of ACh and thus explain the lack of changes in ACh level in this CNS structure.

Résumé. La trémorine a été administrée chez les rats quotidiennement pendant 7 jours à des doses de 7.5, 22.5 ou 30 mg/kg. Les témoins ont montré des mouvements convulsifs proportionnels à la dose de trémorine. Quatre jours après l'arrêt du traitement, l'activité acétylcholinestérasique du cortex cérébral était augmentée. Il est proposé que ce change de l'activité acétylcholinestérasique reflète une utilisation accrue de l'acétylcholine corticale.

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The Rate Limiting Control of Enzymes Monoamine Oxidase and Catechol-*O*-Methyl Transferase in the Foetus and the Adult by Adreno-Cortical Hormones

Early studies suggested that hormones of the adrenal cortex and catecholamines act as a single physiological unit[1,2]. The shifts in the concentration of corticosteroids greatly affect adrenaline and noradrenaline synthesis, release and urinary excretion[3-5]. Studies in the past decade have provided compelling evidence that adrenal cortical steroids play an essential role in the physiology of medullary chromaffin tissue[6,7]. The importance of glucocorticoids in noradrenaline methylation to adrenaline and the induction of enzyme phenylethanolamine-N-methyl transferase (PNMT) have been extensively investigated by WURTMAN and AXELROD[8-11]. Our recent observations indicate that there is a significant rise in output of catecholamine metabolites after hypophysectomy or adrenalectomy[12,13]. To confirm this hypothesis, rabbit foetuses were deprived of their hypophysis by decapitation in utero at the age of 20 days to inactivate the adrenal cortex[14,15]. The activities of enzymes monoamine oxidase (MAO) and catechol-*O*-methyl transferase (COMT) in adrenals, kidneys, paraganglia and lung of decapitated foetuses at the age of 31 days were measured. Adrenalectomy was also performed in new born rats at the age of 0 h and 10 days after their MAO activity was compared with that of normal young rats. To see if adreno-cortical hormones inhibited MAO and COMT in the adult rats, glucocorticoid synthesis was blocked with metopirone[16-18] and the activities of these two enzymes were determined 8 h after metopirone administration.

Materials and methods. White rabbits of New Zealand strain and Sherman rats were utilized throughout the experiments. The female rabbits were made pregnant in our labotarory and verified on the 14th day by palpation. The mothers were operated on the 20th day under nembutal anaesthesia and foetal hypophysectomy by decapitation was performed[14,15]. The maximum number of foetuses decapited from each mother ranged from 2 to 4. Another group of foetuses from unoperated mother also served as controls. 16 decapited foetuses in groups of 8 each were administered with 1.5 mg of hydrocortisone

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