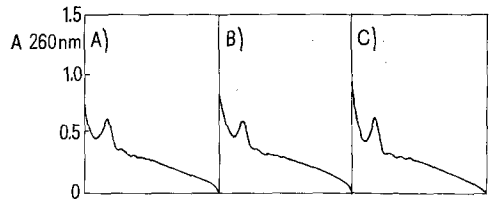

Fig. 1. Sedimentation profiles from the postmitochondrial supernatant of rat brain homogenates. A) normal rat; B) CCl_4-treated rat; C) CCl_3Br-treated rat. Top of the gradient is to the left.

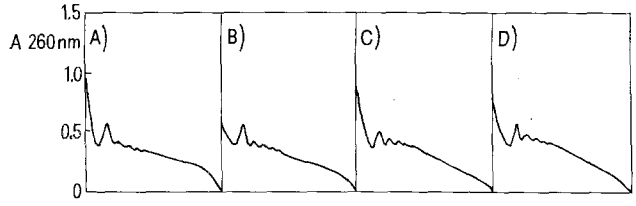

Fig. 2. Sedimentation profiles from the postmitochondrial supernatant of rat brain homogenates. A) CCl_4-treated rat; B) CCl_3Br-treated rat; C) PB + CCl_4-treated rat; D) PB + CCl_3Br-treated rat. Top of the gradient is to the left.

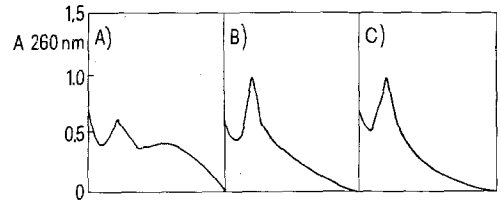

Fig. 3. Sedimentation profiles from the postmitochondrial supernatant of rat liver homogenates. A) normal rat; B) CCl_4-treated rat; C) CCl_3Br-treated rat. Top of the gradient is to the left.

gradient and centrifuged at 200,000 $\times g$ for 40 min in a Spinco SW50 rotor at 0 °C° The extinction profiles at 260 nm were recorded as previously described.

Results. After 2 h of intoxication with CCl_4, as well as with the more toxic CCl_3Br[5], the brain polysomal profile was not different from that of the control group (Figure 1). In addition, these halomethanes failed to modify the polysomal pattern in the brain of animals pretreated with PB (Figure 2). On the other hand, as expected, CCl_4 and CCl_3Br produce polysomal breakdown in the liver, both in normal rats and in PB-treated rats (Figure 3).

Our data show that CCl_4 and CCl_3Br not cause in the brain one of the very typical alteration which they produce in the liver, that is polysomal dissociation. This observation can be explained with the absence in brain tissue of a microsomal system capable of metabolizing foreign drugs. Conversely, since CCl_4 reaches, in brain, a concentration higher than in liver[7], and since polysomal breakdown is a sensitive index of the toxicity by free radicals, our data provide strong evidence that DMES is not present in the rat brain. Finally, our results indicate that neither CCl_4 nor CCl_3Br inhibit protein synthesis per se.

Riassunto. La disaggregazione dei polisomi di fegato di ratto trattato con CCl_4 o CCl_3Br non é riproducibile nei polisomi di cervello, neppure in animali pretrattati con fenobarbital.

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Inhibition of Phosphodiesterase by Dihydroergotamine and Hydergine in Various Organs of the Cat in vitro

The classical phosphodiesterase inhibitors (PEase-I), caffeine and theophylline, are roughly equipotent when tested in vitro on homogenates of various organs[1–3]. However, this is not the case with all substances which reduce phosphodiesterase (PEase) activity.

Lately, numerous papers have appeared reporting different levels of inhibitory activity or organ-specific activity for apomorphine[1], papaverine[1,4,5], 3,4-dihydroxyphenyl-acetic acid[1], quazodine[6], a pyrazolo-pyridine derivative[2] and the bronchodilator 3-acetamido-6-methyl-8-n-propyl-5-triazolo-4, 3-pyrazine[7]. Pichard et al.[8] recently demonstrated in human tissues that the coronary vasodilator dipyridamol inhibits blood platelet PEase 4.5 times more efficiently than the brain enzyme. These authors found that the situation was reversed with tricyclic antidepressants, e.g. nortriptyline, these compounds inhibiting the PEase activity of the brain 3 times more strongly than that of the blood platelets. All these findings suggest that a relationship exists between inhibition of the specific PEase of an organ and pharmacological effect on the organ in question.

Some ergot alkaloids and lysergic acid derivatives are known as PEase-inhibitors. Thus, Kukovetz and Pöch[9] showed that bromo-LSD reduces myocardial PEase activity. Ward and Fain[10] have demonstrated that dihydroergotamine (DHE) inhibits PEase in adipose tissue. We[2,11] noted PEase inhibition in cerebral grey matter with DHE and a large group of analogous compounds. DHE and Hydergine ® (an equimolecular mixture of dihydroergocornine, dihydroergocristine and dihydroergokryptine – PEase-I belonging to the group of compounds mentioned) exert pharmacological effects on the

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Effect of Hydergine and dihydroergotamine on the phosphodiesterase activity of homogenates of various cat organs in vitro

Organ	Inhibitor	Basic value (μmol/30 min × 100 mg fresh tissue ± SD)	% inhibition at a concentration of 10^{-6}	10^{-5}	10^{-4}	$10^{-3}M$
Brain	Hydergine	39.05 ± 6.34	7.5	9.5[a]	15.3[a]	42.7[a]
Heart	Hydergine	15.15 ± 4.02	3.5	− 0.5	6.0	41.0[a]
Lung	Hydergine	3.56 ± 0.99	3.8	9.7	7.0	50.0[a]
Liver	Hydergine	15.30 ± 2.61	− 0.8	− 2.3	− 3.3	36.7[a]
Kidney	Hydergine	20.11 ± 3.99	11.2[a]	1.0	− 1.0	24.5[a]
Brain	DHE	39.05 ± 6.34	5.2	12.2	16.2	43.7[a]
Heart	DHE	15.15 ± 4.02	13.0[a]	3.0	11.0	41.0[a]
Lung	DHE	3.56 ± 0.99	− 7.8	− 8.3	− 3.0	55.0[a]
Liver	DHE	15.30 ± 2.61	4.0[a]	2.7	4.3	44.7[a]
Kidney	DHE	20.11 ± 3.99	− 0.5	0.5	1.7	22.0[a]

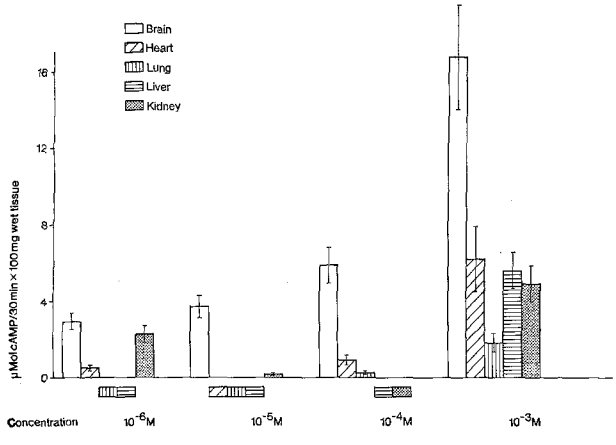
Control value for each organ homogenate = 100. [a] $p < 0.05$ for 2 out of 3 experiments (see text).

CNS. This led us to compare the inhibitory action of these dihydrogenated ergot alkaloids on brain PEase with that of the PEase of other organs. The prime aim of the investigation was to ascertain whether PEase inhibition showed any organ specificity.

Cat organs (heart, kidney, liver and brain) were removed as quickly as possible under pentabarbital anaesthesia, frozen immediately and kept at −20 °C until analysis. The PEase activity of homogenates of these different organs was determined by the method of SUTHERLAND[12] as modified by CHEUNG[13].

DHE and Hydergine were added to the organ homogenates without prior incubation. The concentrations added were selected on the basis of an earlier study[3] of the dependence of PEase-inhibition with DHE on concentration. Concentrations ranging from $10^{-6}M$ to $10^{-3}M$ in powers of 10 were employed for both inhibitors in all organs.

The results were evaluated by means of the Student t-test. 3 separate experiments were carried out, each comprising 6 parallel determinations. A result was considered to differ significantly from the control, if for at least 2 of the 3 separate experiments p was < 0.05. In the Table these values are indicated with an asterisk.



Organ specificity of Hydergine inhibition of phosphodiesterase. Calculated additional remaining amount of cAMP as a result of the in vitro PEase inhibition due to Hydergine in different cat organs. The scale unit of the ordinate is μmol/30 min × 100 mg of fresh tissue ±SD. The symbols shown under the abscissa represent values of less than 0.1 unit. The results obtained with DHE are very similar and therefore not presented.

It will be seen from the Table that only high unphysiological concentrations ($10^{-3}M$) produce uniform pronounced inhibition of PEase in all organs. Lower concentrations, lying near the physiological region (10^{-6} to $10^{-5}M$), reduce PEase activity only in brain homogenate, the degree of inhibition being roughly equal for DHE and Hydergine. This result is in good agreement with the findings of our previous study[11], in which the separate components of Hydergine were compared with DHE. The degree of inhibition with Hydergine is slightly less than the mean value for its separate components.

Ignoring the effect ot higher cAMP concentrations as a consequence of the law of mass action, the concentrations of intact cAMP per unit weight of fresh tissue resulting from inhibition clearly show (Figure) that at physiological dose levels DHE and Hydergine induce an accumulation of cAMP only in brain homogenate. The rise in concentration of the second messenger activates the corresponding protein kinases (PKase), i.e. the information transmitted by cAMP should be reinforced by PEase-I. In cytochemical and electron microscopic studies Greengard et al.[14–16] have demonstrated that brain PEase is to be found mainly in postsynaptic nerve endings. Since these structures also contain large amounts of adenylcyclase and PKase, it is highly probable that the cAMP system plays a part in synaptic transmission brought about by noradrenaline and dopamine. How the information is passed on to nerve tissue by PKases is still largely unknown.

The organ-specific inhibition of PEase by DHE and Hydergine noted in this study could be due to a different isoenzyme pattern in brain tissue. This question has been investigated by MONN and CHRISTIANSEN[17] and by CAMPBELL[18] using special electrophoretic methods. Both authors reported the presence of specific PEase isoenzymes occurring exclusively in the CNS. CHEUNG[19, 20]

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found that brain PEase differs from PEase of other organs in that a considerable fraction is insoluble. This fraction can be rendered soluble by treatment with Triton X-100. One possible explanation of our findings is that PEase-inhibition has an effect on this insoluble enzyme or interacts with the proteinlike inhibitor reported by CHEUNG[21].

A more accurate insight into the causal relationships discussed in this paper will emerge from further work on isolated isoenzymes and total PEase preparations from various organs.

Zusammenfassung. Die Hemmung der cAMP-Phosphodiesterase in verschiedenen Organen der Katze durch Hydergin und DHE wurde in vitro bestimmt. In Konzentrationen von $10^{-4}M$ bis $10^{-6}M$ wurde ein Hemmeffekt nur im Gehirn beobachtet. Diese Wirkung wird besonders deutlich, wenn man das infolge der Enzymhemmung nicht umgesetzte cAMP berechnet.

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Ontogenetic Differences between Acetyl and Butyrylcholinesterase Isozymes in the Chick Embryo Cerebellum

Multiple forms of acetylcholinesterase (AchE: E.C.3.1.1.7) and butyrylcholinesterase (BuchE: E.C.3.1.1.8) have been detected in neural tissue and the serum of many vertebrate species[1–5]. Total AchE synthesis increases during periods of accelerated brain development[6], with the appearance of synaptic vesicles, and with bioelectric activity in the spinal cord[7]. Rapid increases of total AchE and BuchE content are known to occur in the chick cerebellum between the 10th and 14th day of incubation[8]. This interval corresponds to the first occurrence of extensive body movements by the chick embryo[9]. In *Ambystoma punctatum* significant increases in AchE also first occur with motile behavior[10,11]. Thus, AchE and BuchE isozymes have been considered as indices of vertebrate neural and neuralmuscular development. This report describes changes in AchE and BuchE isozymes during this period of rapid neural development in the chick embryo cerebellum by the techniques of isoelectric focusing.

Embryos were obtained from a genetically homogenous strain of White Leghorn chickens and incubated under standard conditions. Tissues were collected from 10- and 14-day-old embryos and prepared for enzyme analysis[12,13]. A 110 ml isoelectric focusing column was prepared according to manufacturer's instructions[14]. Twice the recommended amount of Ampholytes (pH 3–10) were used to increase protein solubility with 0.1 ml of the supernatant being added after the column was partially filled. Conditions of 4 °C, 600 V, and 10 mA were maintained with the cathode at the top of the column. When the current stabilized at 1–2 mA the column was emptied in 40 drop fractions. The pH of each fraction was recorded and then adjusted to pH 8.0 to eliminate any pH effect on the enzyme reactions. Separate experiments demonstrated that Ampholytes had no effect on the basic enzyme reaction and that different homogenizing media[12] did not significantly alter the pH gradient. 6 replicates were analyzed for each age. Comparisons were made between the isoelectric point of each isozyme within an isozyme complex (either AchE and BuchE) by F-ratió analysis and DUNCAN's multiple range test and between isozyme complexes (AchE and BuchE) with the student *t*-test.

Three isozymes were resolved for both AchE and BuchE in the 10-day-old cerebellum (Figure 1). The activities between each isozyme complex were significantly different ($P \leq 0.05$). AchE isozymes had isoelectric points similar to those of the BuchE isozymes. Analysis within isozyme complexes demonstrated that the isoelectric points of the different isozymes of each enzyme were significantly different ($P \leq 0.001$).

Five isozymes of AchE and BuchE were detected in the 14-day-old cerebellum (Figure 2). Enzymes activities were significantly different ($P \leq 0.05$) between AchE and BuchE. The isoelectric points between AchE and BuchE isozymes I, II, and III were significantly different ($P \leq 0.01$); AchE and BuchE isozymes IV and V had

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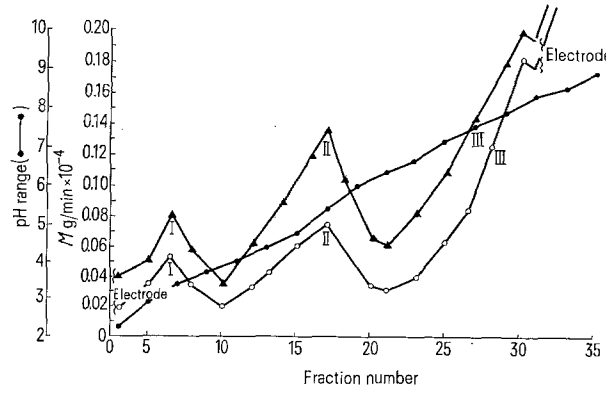

Fig. 1. 10-day-old cerebellum isoelectric focusing of AchE and BuchE isozymes. Key: ●, pH; ▲, AchE; and ○, BuchE.