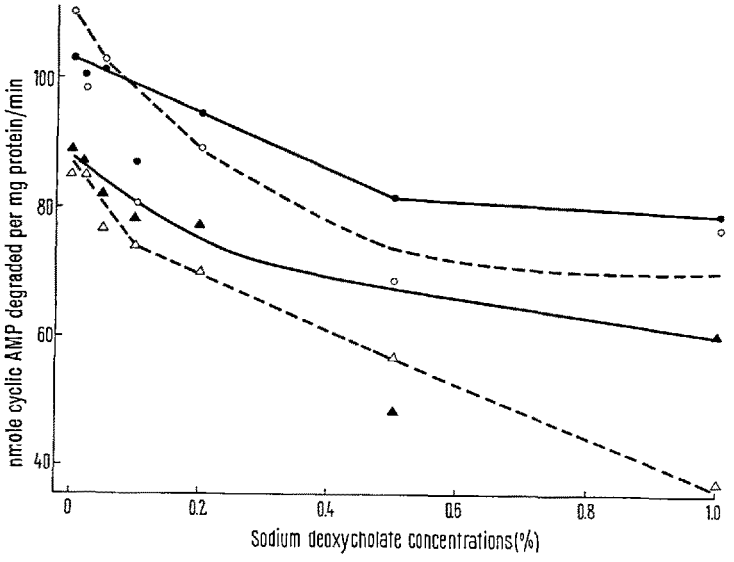

2. Effect of increasing DOC concentrations on phosphodiesterase activity. The fractions are: ●——●, ACTH, particulate; ▲——▲, ACTH, supernatant; ○- - -○, control, particulate; △- - -△, control, supernatant.

The increase of PD activity in the presence of DOC after ACTH administration in vivo suggests that there is a hormonal effect on the enzyme activity. It is very likely that ACTH stimulates a de novo synthesis of PD in the adrenals, an effect similar to that ascribed to insulin[8]. The newly synthesized enzyme, however, is not accessible to the substrate. The exact site where de novo synthesis takes place and the nature of barrier to the substrate are open to speculation.

Résumé. Le désoxycholate de soude inhibe fortement l'activité de la phosphodiestérase surrénalienne du rat. Par contre, l'administration de l'ACTH in vivo augmente cette activité en présence du déoxycholate. In vitro, chez le rat traité préalablement par le l'ACTH, cette activité est moins inhibée par le désoxycholate dans la fraction particulière comme dans la fraction surnageante. L'action de l'ACTH joue donc un rôle régulateur de la phosphodiestérase.

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The Effect of Dihydroergotamine on the Phosphodiesterase Activity of Cat Grey Matter

Since the investigations of SUTHERLAND[1-3] it has been assumed that cyclic adenosine monophosphate (cAMP) fulfils the function of a second messenger in certain hormone effects. Accordingly the statistical life of cAMP in the cell appears to be a measure of the activity of many hormones. SUTHERLAND was the first to observe the activation of adenyl cyclase (ACase) by adrenaline. LANGAN[4], MIYAMOTU, KUO and GREENGARD[5] pointed out the importance of cAMP for central nervous RNA metabolism and long-term memory. The concentration of the second messenger depends upon phosphodiesterase (PEase), the enzyme which inactivates cAMP by cleavage to 5'-AMP, as well as upon adenyl cyclase. The cleavage proceeds by nucleophilic substitution at C3 of the ribose moiety. The liberation occurs in the presence of Mg^{++} in order to preserve the steric configuration of the enzyme molecule.

All substances affecting one of these enzyme systems thus influence the concentration of intracellular cAMP. PEase inhibitors, such as methylxanthines, caffeine, theophylline, papaverine and 2-bromlysergic acid diethylamide (BOL 148)[6,7] investigated by KUKOVETZ and PÖCH[8] reinforce the effect of cAMP in a manner similar to the catecholamines. The activity of various inhibitors differs from organ to organ, as KUKOVETZ and PÖCH[8] recently showed in the case of papaverine. WILLIAMS[9] found a similar state of affairs in the grey matter. He noted that theophylline had only a slight PEase-inhibitory effect on cerebral cortex, as compared with other organs.

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The effect of various inhibitors of phosphodiesterase activity

Inhibitor	Control[a] series	Inhibitor[a] series	Inhibition with respect to control (%)	p-value	Inhibition in respect to caffeine = 1
Caffeine	44.0 ± 3.0	32.0 ∓ 3.6	27	0.0125	1.00
Theophylline	44.0 ± 3.0	38.0 ± 2.1	14	0.08	0.52
Papaverine	44.0 ± 3.0	48.5 ± 7.0	−10	0.30	−0.37
DH-Ergotamine	42.4 ± 8.4	30.2 ± 3.5	29	0.10	1.07

[a] µmol undergoing cleavage ± S.D. Concentration of all inhibitors $2.5 \times 10^{-5} M$. 6 experiments calculated for the activity of 100 mg dry brain tissue (grey matter, cat). Statistical evaluation by the Student 's t-test. Controls always comprised the same starting material as the inhibitor series.

The present paper describes the effect of dihydroergotamine (DHE) on the PEase activity of grey matter. No similar investigation has so far been reported.

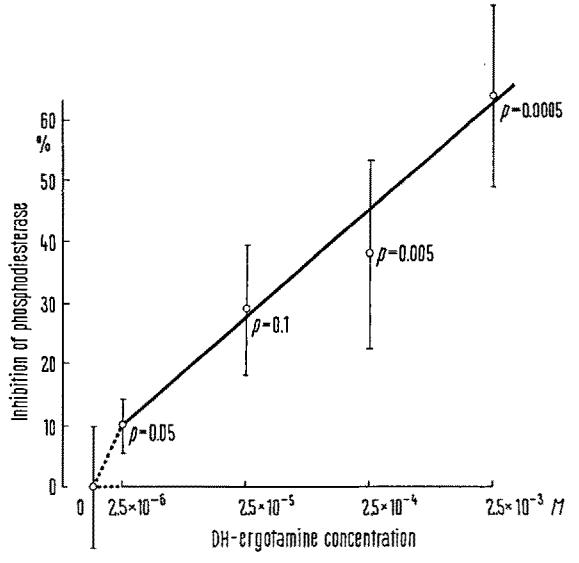
Various groups of 1 to 3 cats were employed. The brain was isolated under shallow pentobarbitone anaesthesia. The grey matter was dissected out under histological control using a modified LOWRY technique. (The isolation of the grey matter and the histological control procedure were performed in the morphological laboratory of our Department.)

The tissue was carefully freeze-dried. An interval of 20–30 sec elapsed between interruption of the circulation and freezing of the brain tissue in isopentane (−78 °C). The tissue was incubated by a modification of WILLIAMS's method[9], and chromatographic separation was effected by a method based on that of KRISHNA[10]. The concentration of cAMP in our material was so high that radioactive labelling proved unnecessary. Following the complete chromatographic separation the decrease in concentration of cAMP and the increase in concentration of 5'AMP have been shown to be a good measure for the estimation of the PEase-activity. We determined the concentration of adenosine nucleotides spectrophotometrically at 260 nm.

In the first part of our expriments we compared the inhibitory effects of caffeine, theophylline, papaverine and dihydroergotamine in a uniform concentration of $2.5 \times 10^{-5} M$. The results are shown in the Table.

In the second part of our experiments, we determined PEase inhibition as a function of various DHE concentrations. The results are shown in the Figure. As indicated in the Table the inhibitory effect of DHE was of the same order of magnitude as that of methylxanthine. Unlike MARKWARDT[11], who noted maximum PEase inhibition in blood platelets with papaverine, we did not observe any inhibition in cerebral cortex at the papaverine concentrations. PÖCH and KUKOVETZ[6] likewise found relatively weak enzyme inhibition with papaverine in whole brain.

Our results show that PEase inhibition depends in roughly linear fashion upon the logarithm of the DHE concentration (Figure). As for other inhibitors, inhibition of PEase activity by DHE appears to differ from organ to organ. Thus VOGEL et al.[12] failed to find any inhibition with DHE on the lipolytic system of fat cells.



The effect of various DHE concentrations on the phosphodiesterase activity of cat grey matter extract. Inhibition with respect to the control is plotted as a function of the logarithm of the dihydroergotamine concentration. Control value: 42.4 ± 8.4 µmol cAMP per 100 mg dry grey matter in 30 min. Variation and p value by the Student's-t-test are also entered.

Zusammenfassung. Es wurde die Hemmung der Phosphodiesterase PEase durch DHE untersucht. Die Inhibition durch DHE liegt in derselben Grössenordnung wie diejenige der Methylxanthine. Die Dosisabhängigkeit der PEase-Hemmung durch DHE ist nahezu linear zu den in Dekaden aufgetragenen Konzentrationen.

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