The Regulatory Role of Phosphodiesterase in Adenosine Cyclic 3′, 5′-Monophosphate Mediated ACTH Action

The mediation by adenosine cyclic 3′, 5′-monophosphate in adrenocorticotrophic hormone (ACTH) action is now very well documented[1]. There is a stimulatory effect of the hormone on adrenal adenyl cyclase[2]; ACTH effect on cAMP-specific phosphodiesterase (PD) is not clear[3,4]. A stimulatory effect is revealed in the present study by using sodium deoxycholate (DOC).

3 mature female white rats were each injected i.m. with 0.2 U ACTH in 0.33 ml saline. The same volume of saline was given to each of 3 control rats. After 1 h, the rats were decapitated. Adrenals were removed, freed of adipose tissue and quickly frozen at –60 °C. Adrenals of each group were homogenized in 3 ml of 0.25 *M* sucrose in 0.1 *M tris*-HCl buffer, pH 7.8. The homogenates were centrifuged at 30,000 × *g* for 30 min at 4 °C. The pellets were resuspended in 3 ml of homogenization medium and divided into 2 portions. To 1 portion was added DOC to a final concentration of 0.38%. The same volume of water was added to tha other portion. These 4 resuspensions were then centrifuged at 30,000 × *g* for 30 min, and the pellets were resuspended in homogenization medium to the initial volume. 0.1 ml aliquots from each of the 10 enzyme preparations were assayed for PD activity. The assay mixture contained, in a final volume of 0.25 ml after enzyme addition, 2 m*M* cAMP, 0.166 μC/ml ^{3}H-cAMP, 2 m*M* $MgCl_2$ and 50 m*M tris*-HCl buffer, pH 7.8. Incubation lasted for 15 min at 37 °C. The reaction was terminated by boiling in water for 3 min. Denatured protein was sedimented at 5,000 × *g* for 15 min. 0.2 ml of the supernatants was applied to Whatman 3 mm paper and developed downwards for 20 h with 95% ethanol-1 *M* ammonium acetate, 2.9 m*M* EDTA, pH 8.0 (70/30, v/v). UV-absorbing spots corresponding to cAMP were cut, soaked in 2 ml 0.1 *N* HCl overnight and counted in 10 ml BRAY's scintillant[5] in a Nuclear Chicago Mark I liquid scintillation counting system. Protein contents of the enzyme preparations were determined by the method of LOWRY[6].

The results (Figure 1) indicate that ACTH administration in vivo does not have a marked effect on enzyme activities of both particulate[7] and supernatant fractions. The ratio of activities fo the 2 fractions appears to be unaffected, with 2.64 for the experimental group against 2.45 for the control. The ratio of activities of resuspension to wash, however, indicates a 45% decrease after ACTH treatment. ACTH may thus render the membrane bound enzyme more susceptible to solubilization upon washing.

PD activity is inhibited in 3 of the 4 enzyme preparations treated with DOC. The exception is the resuspension from the experimental group which shows an increase by 2.5 folds over the same enzyme preparation without DOC. In addition, DOC increases the ratio of activities of resuspension to wash, but the increase is greater in the experimental group than in the control. The overall effect is that, after DOC treatment, enzyme activity of particulate fraction from the experimental group exhibits an increase while that from the control shows a decrease. The ratio of particulate to supernatant activity is thus increased in the experimental group but is decreased in the control.

The increase of PD activity in resuspension from ACTH-treated rats is not due to a stimulatory effect from the residual DOC left after washing. Particulate and supernatant fractions were preparated from adrenal homogenates from 3 experimental and 3 control rats as described above. 0.1 ml aliquots of the 4 enzyme preparations were assayed in the presence of various concentrations of DOC in the same assay mixture.

The effect of DOC on PD activity is an outright inhibition from low to high concentrations (Figure 2). In the absence of DOC, ACTH administration in vivo does not appear to affect enzyme activities of both particulate and supernatant fractions. However enzyme preparations from the experimental group, both the particulate and the supernatant fractions, are distinctly less inhibited by DOC than the corresponding preparations from the control.

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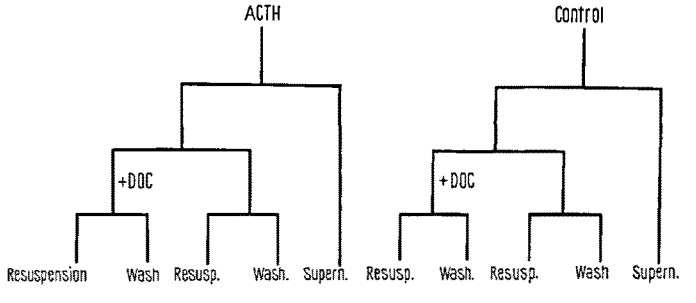
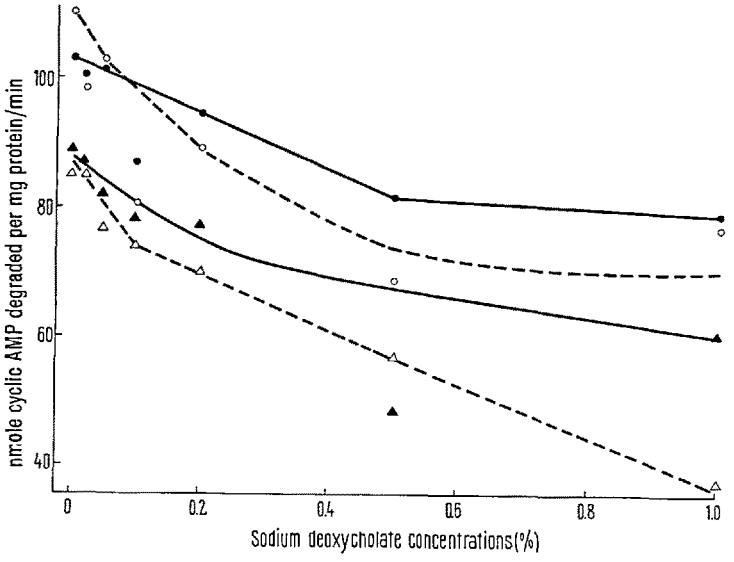


Specific activity	4.76	1.32	1.94	2.00	1.49	2.30	0.80	2.63	1.48	1.68
Resusp. / Wash		3.60		0.97			2.80		1.77	
Particulate / Supernat		4.08		2.64			1.85		2.45	

1. ACTH and DOC effect on phosphodiesterase activity. Each value is the average of 2 duplicate samples. Enzyme activity = μmole cAMP degraded/mg protein, 15 min.



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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