PHOSPHODIESTERASE ACTIVITY AND THE POTENTIATION BY THEOPHYLLINE OF ADRENOCORTICOTROPHIN STIMULATED STEROIDOGENESIS AND ADENOSINE 3',5'-MONOPHOSPHATE LEVELS IN ISOLATED RAT ADRENAL CELLS.

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Summary. Corticosterone production and adenosine 3',5'-monophosphate levels in collagenase prepared isolated rat adrenal cells have been measured in response to adrenocorticotrophin in the presence and absence of theophylline. Theophylline (1mM) was found to potentiate the steroidogenic effect of submaximal concentrations of adrenocorticotrophin. This concentration of theophylline was without effect on protein synthesis in this system. Potentiation of adrenocorticotrophin stimulated adenosine 3',5'-monophosphate levels was also observed in the presence of theophylline (0.5 and 1.0mM). Phosphodiesterase activity in collagenase prepared adrenal cells was 67% of that in intact glands, while the activity in trypsin prepared cells was 37% of that in intact glands.

The methylxanthines, such as theophylline and caffeine, are noted for their inhibitory effect on phosphodiesterase (1). This enzyme inactivates adenosine 3',5'-monophosphate (cyclic AMP), believed to be the intracellular mediator in the steroidogenic effect of adrenocorticotrophin (ACTH) in the adrenal (2, 3, 4). For many protein hormones the potentiation of their effect by methylxanthines has provided good circumstantial evidence that these hormones exert their effects via cyclic AMP (1). However, this important criterion has not yet been clearly established for the ACTH steroidogenic effect.

Previous workers have found methylxanthines to have no potentiating effect on ACTH stimulated steroidogenesis either in the quartered adrenal gland (5) or in trypsin prepared isolated adrenal cells using theophylline (6) or caffeine (7). Moreover, Carchman et al. (8) have reported that although the isolated cat adrenal perfused in situ responded to theophylline with increased basal and ACTH stimulated

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cyclic AMP levels, the corticosterone release was not enhanced. Halkerston et al. (5) explained the lack of effect of theophylline in the quartered gland by its observed inhibition of protein synthesis, considered to be essential for steroidogenesis (9). Kitabchi et al. (7) have studied the activity of cyclic AMP phosphodiesterase in isolated adrenal cells prepared by trypsin digestion, and found it to be very much lower than the activity in whole adrenals. They explain the observed lack of a potentiating effect by caffeine in their trypsin prepared adrenal cells by the low phosphodiesterase activity in these cells.

Methylxanthines, however, do exhibit competitive inhibition of partially purified phosphodiesterase from whole adrenal tissue (10), and studies in vivo have demonstrated that in the hypophysectomised rat both the basal and the ACTH stimulated corticosterone secretion were increased by acute theophylline administration (11). In a recent preliminary report (12) a stimulatory effect by some phosphodiesterase inhibitors on ACTH induced steroidogenesis by collagenase prepared isolated adrenal cells has been noted. The present communication details the stimulatory effect of theophylline on isolated adrenal cells prepared by collagenase disaggregation (13). In order to clarify its role in this system, phosphodiesterase activity in collagenase prepared cells and in trypsin prepared cells (14) has been compared with the activity in adrenal tissue.

Part of this work has been reported in preliminary form (15).

Materials and Methods

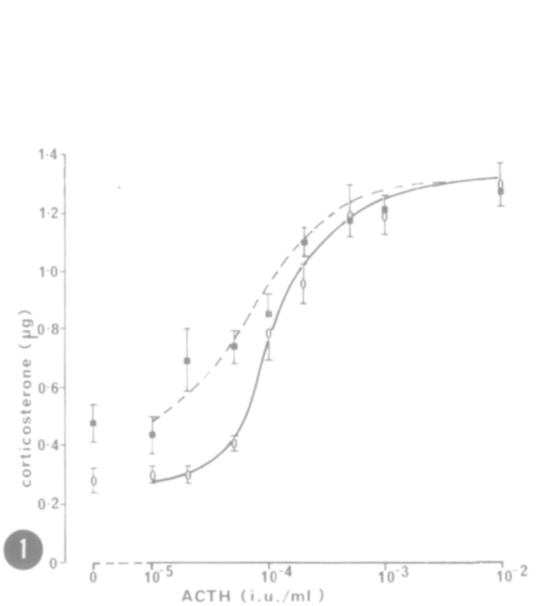
Isolated rat adrenal cells were prepared by collagenase dispersion of decapsulated glands (13). Incubation conditions are shown in the figure legends. After incubation of the cell suspensions for 1h, corticosterone accumulated in cells and medium was measured by a fluorometric assay, adapted (16) from the method of Silber et al.

(17). Theophylline tested in this assay had no effect on the fluorescence of a range of standard corticosterone samples. Cyclic AMP levels were measured in cells and medium by a method (18) which includes chromatography of the samples on Dowex AG 50W-X8 (100-200 mesh; BioRad Labs, Richmond, Calif.), followed by a protein binding assay based on the method of Brown et al. (19). Phosphodiesterase activity was assayed by the method of Sams and Montague (20), which measures the rate of breakdown of [8-^{3}H]adenosine 3',5'-monophosphate (20.7Ci/mmol; The Radiochemical Centre, Amersham, Bucks, U.K.) to [^{3}H]5'-AMP and then to [^{3}H]adenosine. Protein synthesis by the cells was estimated from the incorporation of L-[1,2-^{3}H]leucine (1Ci/mmol; The Radiochemical Centre, Amersham, Bucks, U.K.) into trichloroacetic acid-precipitable protein. Protein content was estimated by the method of Lowry et al. (21) and isolated cells were washed with protein-free medium before assaying for protein content. Trypsin disaggregated adrenal cells were prepared as described by Lowry et al. (14).

ACTH (Acthar Corticotrophin) was a gift from Armour Pharm., Eastbourne, England, and theophylline hydrate (m.p.269^{o}-274^{o}C) was purchased from B.D.H. Ltd., Poole, U.K.

Results and Discussion

The effect of theophylline (1mM) on the ACTH stimulated steroidogenesis of collagenase prepared isolated cells is shown in Fig. 1. At submaximal concentrations of ACTH an increase in steroid production was found in the presence of theophylline. No increase in steroid production was seen at concentrations of ACTH maximal for steroidogenesis. This is reasonable as it has been shown (18, 22) that cyclic AMP levels in the adrenal cell continue to rise with increasing concentrations of ACTH, in excess of that needed to elicit maximum steroidogenesis. Thus any extra increase in cyclic AMP levels caused by theophylline at high ACTH concentrations would have no effect on



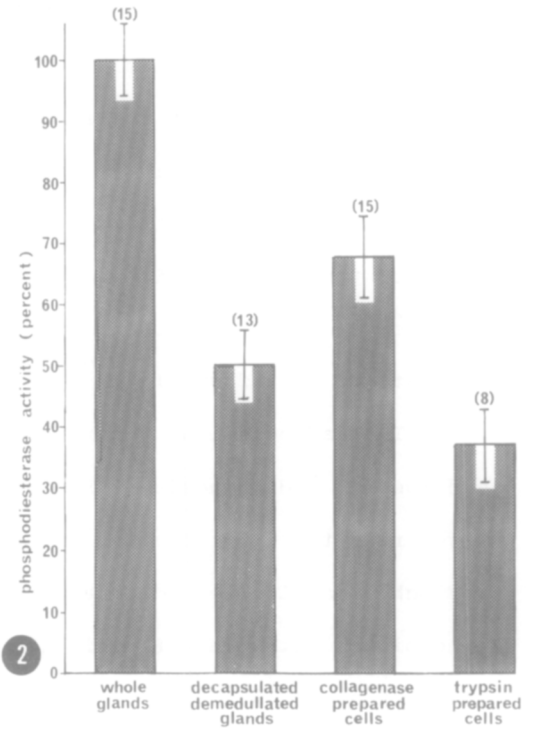


Fig. 1. Corticosterone production by collagenase prepared isolated adrenal cells treated with a range of ACTH concentrations in the absence (0——0) and presence (■- - - -■) of theophylline (1mM). Incubations were normally in 0.5ml, containing the cells isolated from the equivalent of quarter of an adrenal. Results are expressed as the amount of corticosterone produced by the cells isolated from the equivalent of two adrenals. Error bars represent S.E.M. for six experiments, in each of which incubations were in duplicate.

Fig. 2. Phosphodiesterase activity in adrenal tissue after different treatments. The activity found in whole adrenal glands was taken as 100% and results are expressed in relation to this value, which corresponds to 21.9 nmoles of cyclic AMP hydrolysed/10min/mg protein. Error bars represent S.E.M. and (n) the number of estimations.

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the maximal rate of steroidogenesis. The doses of theophylline used in these experiments were shown to have no significant effect on protein synthesis in this system. Of the [^{3}H]leucine incorporated by cells alone, 97% was incorporated by cells in the presence of 0.5mM theophylline and 91% by cells in the presence of 1mM theophylline.

Cyclic AMP levels were measured in cell suspensions treated with a range of ACTH concentrations in the presence and absence of two

Theophylline (mM)	Cyclic AMP (ng)			
	0 i.u. ACTH/ml	$1x10^{-5}$ i.u.ACTH/ml	$1x10^{-3}$ i.u. ACTH/ml	$1x10^{-2}$ i.u. ACTH/ml
Expt. I				
0	0.72, 0.52	0.37, 0.28	6.09, 7.86	-
0.5	0.70, 0.77	1.36, 1.19	22.50, 22.17	-
Expt. II				
0	1.74, 1.33	1.26, 0.92	8.75, 5.58	70.72, 105.68
1.0	1.61, 1.56	1.23	18.29	150.24, 120.85

Table 1: Effect of theophylline on cyclic AMP levels in collagenase prepared isolated adrenal cells and medium

Incubations (1.0 ml) contained the cells isolated from the equivalent of one adrenal. Results are expressed as levels of cyclic AMP in cells isolated from the equivalent of two adrenals. Results from duplicate incubations are shown. At least triplicate assays were performed on each sample.

doses of theophylline. The results of these experiments are shown in Table 1. At 1 x 10^{-5} i.u. ACTH/ml a potentiating effect by theophylline was discernible although at low concentrations of ACTH this effect was not consistently observed. However, from previous studies (18, 22) it is clear that only very small changes in overall cyclic AMP levels are necessary to produce significant changes in corticosterone production. Thus the overall changes in cyclic AMP levels associated with the increase in steroid production shown in Fig. 1 may be below the limit of detection of assay systems for cyclic AMP currently available. On the other hand, at higher concentrations of ACTH (1 x 10^{-3} i.u./ml and above) a marked and consistent potentiation of cyclic AMP levels was noted. Thus theophylline potentiates the ACTH effect on both corticosteroidogenesis and cyclic AMP levels, in collagenase prepared isolated adrenal cells.

Kitabchi et al. (7) explain the lack of a potentiating effect by caffeine in their system in terms of the low phosphodiesterase activity observed in their trypsin prepared cells (4.5% of the activity found in whole adrenal homogenates). It was thus considered important to compare the phosphodiesterase activity in the collagenase prepared cells used in this study (in which capsular and medullary cells have been eliminated (13) by the preparative procedure) with that in decapsulated-demedullated adrenal tissue, as well as that in trypsin prepared isolated cells and in whole adrenal glands. The results of this comparison are shown in Fig. 2. The higher activity observed in collagenase prepared cells when compared with decapsulated-demedullated tissue may be explained either by the partial elimination of certain cell types (e.g. zona reticularis cells) from the cell suspension, or by elimination of intercellular protein. Examination of the trypsin prepared cells revealed 37% of that phosphodiesterase activity found in whole adrenal glands, and 70-80% of the activity found in decapsulated-demedullated tissue. As these results are in direct contrast to the findings of Kitabchi et al. (7), it would appear that the precise method of preparation of the isolated cells, as well as the disaggregating enzyme used, may have a significant effect on the characteristics of the cell suspension produced.

These results now establish an important criterion for cyclic AMP involvement in the steroidogenic action of ACTH on the adrenal, and indicate that the system used for these studies is particularly well suited to the further investigation of cyclic AMP involvement.

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Addendum

During preparation of this manuscript a potentiation by 1-methyl, 3-isobutylxanthine of the stimulatory effect of ACTH on adrenal quarters and in vivo has been published (23).

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