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THE ACTION OF ADENOSINE ON STEROIDOGENESIS IN ISOLATED RAT ADRENOCORTICAL CELLS

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SUMMARY

The regulatory role of adenosine in a number of tissues has been the subject of recent investigations [1, 5, 7, 14]. In adrenal tumour cells the nucleoside appears to stimulate adenylate cyclase at an external site in a manner that is antagonised by theophylline [14]. The present report describes a comparison of the effects of theophylline, adenosine and related compounds on steroid production by normal isolated adrenal cells. Neither adenosine or theophylline affected basal steroidogenesis over a wide concentration range. However both compounds markedly enhanced ACTH-stimulation of steroid production without increasing maximal output. The agents exhibited additive effects when used in combination, suggesting that they acted at a common site. It is proposed that in normal adrenal cells adenosine does not stimulate adenylate cyclase but inhibits cyclic-3',5'-AMP phosphodiesterase leading to increased steroid production in the presence of ACTH. These findings question the validity of using adrenal tumour cells as a model for studying the action of ACTH.

1. INTRODUCTION

In adipose tissue low concentrations (approx. 10^{-5} M) of adenosine inhibit both basal and hormone-stimulated lipolysis and cyclic AMP accumulation [1–3]. Also, incubation of fat cells with adenosine deaminase enhances norepinephrine stimulation of both these processes, leading to the suggestion that endogenous adenosine is a feedback regulator of lipolysis [1]. The adenylate cyclase activity of plasma membrane preparations from rat adipocytes and liver and guinea pig lung is also inhibited by low concentrations of adenosine [2, 4–6].

By contrast, in brain tissue and tumours derived therefrom, adenosine appears either to directly stimulate adenylate cyclase activity or to enhance stimulation of the enzyme by norepinephrine [7–10]. The methylxanthines, caffeine and theophylline, competitively antagonise the effects of adenosine [7–9], whereas papaverine (a non-methylxanthine cyclic AMP-phosphodiesterase inhibitor) enhances the effect of the nucleoside in intact tissue [10].

Additionally, high concentrations (approx. 10^{-2} M) of adenosine, as well as theophylline, inhibit the cyclic AMP-phosphodiesterase activity of many tissues [11].

The action of adenosine on normal adrenal cells is not known. An early report suggested that in quartered adrenal glands the nucleoside augmented the steroidogenic response to ACTH [12]. Low theophylline concentrations (1 mM) slightly enhanced ACTH-stimulated steroidogenesis in halved adrenal glands while a higher methylxanthine concentration (10 mM) caused inhibition of the maximal steroid output [13]. This inhibitory effect was thought to be due to decreased protein synthesis [13].

More recent studies on the effect of adenosine on adrenal tumour cells indicate that adenosine and related nucleosides stimulate steroidogenesis and adenylate cyclase activity in the absence of ACTH [14, 15]. Both these effects are antagonised by theophylline [15]. Thus it appears that in adrenal tumor cells adenosine acts at an external regulatory site which is capable of interacting with adenylate cyclase in an analogous manner to the effect of the nucleoside on brain tissue.

The present report compares the action of adenosine and related compounds on corticosterone production by normal isolated adrenocortical cells.

2. MATERIALS AND METHODS

Adenosine, AMP, ADP, ATP, cAMP, cGMP and theophylline were obtained from Sigma (London) Ltd. Collagenase (0.15 μ/mg) was from Boehringer Corporation (London) Ltd. and ACTH was the World Health Organisation IIIrd International Standard. Human Serum Albumin was a gift from Dr. W. D'A. Maycock of the Lister Institute, Elstree, Herts., U.K.

Isolated rat adrenocortical cells were prepared by collagenase dispersion of decapsulated glands as previously described [16]. Cells (75,000/tube) were incubated (30 min., 37°C: 0.5 ml) in the presence of the various effectors noted in the figure legends and their corticosterone production was assayed fluorimetrically [16]. Experiments were performed in a randomised block format to eliminate bias due to systematic error.

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Table 1. The corticosteroidogenic capacity of adenosine, theophylline, ACTH and various purine nucleotides on rat adrenocortical cells

Agent	Concentration (mM)	μg Corticosterone rat h ± S.E.M.
Adenosine	0.02	0.22 ± 0.05
	0.20	0.20 ± 0.01
	2.0	0.17 ± 0.02
AMP	0.02	0.17 ± 0.01
	0.20	0.15 ± 0.01
	2.0	0.21 ± 0.02
ADP	0.02	0.17 ± 0.04
	0.20	0.22 ± 0.01
	2.0	0.26 ± 0.01
ATP	0.02	0.16 ± 0.02
	0.20	0.16 ± 0.01
	2.0	0.22 ± 0.04
cAMP	0.02	0.24 ± 0.02
	0.20	0.31 ± 0.04
	2.0	0.50 ± 0.05
cGMP	0.02	0.20 ± 0.01
	0.20	0.29 ± 0.04
	2.0	0.30 ± 0.01
Theophylline	0.02	0.19 ± 0.01
	0.20	0.23 ± 0.01
	2.0	0.21 ± 0.02
ACTH	1×10^{-3}	1.40 ± 0.04
Concentration	5×10^{-5}	0.67 ± 0.14
(IU/ml)	0	0.21 ± 0.03

3. RESULTS

Of the agents tested, up to concentrations of 2 mM, only cyclic AMP influenced basal corticosterone secretion (Table 1). To measure the effects of the agents on hormone-stimulated steroidogenesis, a dose of ACTH was chosen (5×10^{-5} IU/ml) which elicited a sub-maximal response. Comparison of the corticosterone secretion induced by such ACTH concentrations, with that produced in the presence of both ACTH and the effectors shows clear enhancement of the cell response by all agents except cyclic GMP and ADP (Table 2). The most potent agents were

Table 2. The effect of adenosine, theophylline and various purine nucleotides on corticosteroidogenesis induced by sub-maximally effective concentrations of ACTH (5×10^{-5} IU/ml) in rat adrenocortical cells

Agent	Concentration (mM)	μg Corticosterone/rat/h ± S.E.M.
Adenosine	0.02	1.0 ± 0.11
	0.20	1.21 ± 0.08
	2.0	1.16 ± 0.02
AMP	0.02	0.89 ± 0.12
	0.20	0.91 ± 0.04
	2.0	1.14 ± 0.04
ADP	0.02	0.88 ± 0.05
	0.20	1.07 ± 0.03
	2.0	0.97 ± 0.14
ATP	0.02	0.87 ± 0.09
	0.20	0.74 ± 0.09
	2.0	1.28 ± 0.05
cAMP	0.02	0.88 ± 0.02
	0.20	0.94 ± 0.01
	2.0	1.31 ± 0.01
cGMP	0.02	0.79 ± 0.01
	0.20	0.97 ± 0.02
	2.0	0.71 ± 0.03
Theophylline	0.02	0.86 ± 0.12
	0.20	1.13 ± 0.04
	2.0	1.30 ± 0.01
ACTH	0	0.27 ± 0.03
Concentration	5×10^{-5}	0.85 ± 0.11
(IU/ml)	1×10^{-3}	1.43 ± 0.01

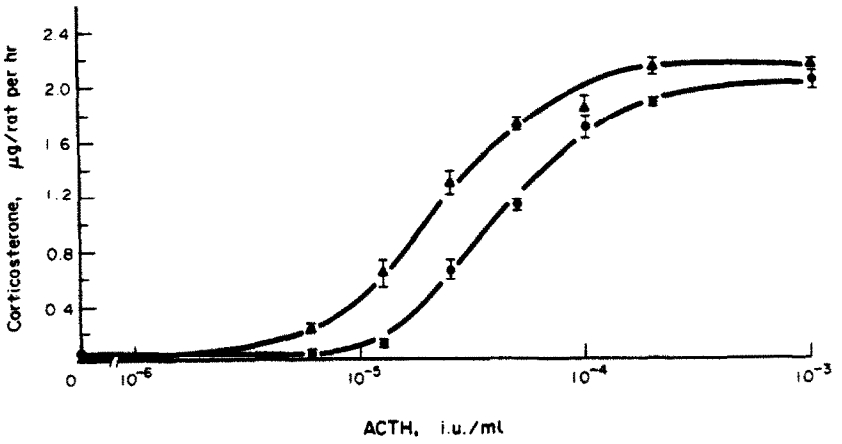

Fig. 1. ACTH dose-response curve in the absence (●) and in the presence of adenosine (0.2 mM: ▲).

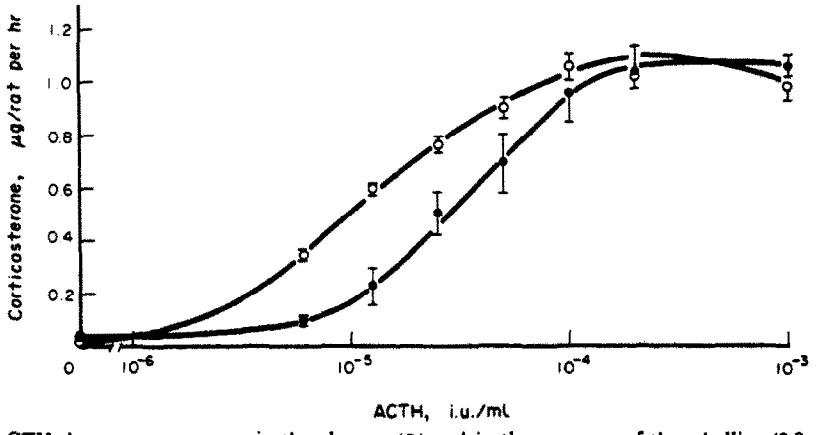

Fig. 2. ACTH dose-response curve in the absence (●) and in the presence of theophylline (2.0 mM: ○).

adenosine and theophylline, the former giving a 30% enhancement at 0.2 mM and the latter giving a 53% enhancement at 2 mM. Concentrations of effectors which were lower than those indicated in Tables 1 and 2 were without effect on either basal or sub-maximally stimulated steroidogenesis.

The effects of adenosine and theophylline were compared on the full range of effective ACTH concentrations. Adenosine (0.2 mM) and theophylline (2.0 mM) reduce the $K_{0.5}$* value by 2- and 3-fold respectively (cf. Figs 1 and 2). Neither agent affects basal or maximal levels of corticosteroidogenesis.

Having established the independent effects of adenosine and theophylline, their combined influence on ACTH-stimulated steroidogenesis was investigated. Combinations of both maximally and submaximally effective concentrations of adenosine (0.2 mM, 0.02 mM) and theophylline (2.0 mM, 0.2 mM) respectively were compared. The effects of maximally effective concentrations of the two agents, alone or in combination, on the stimulation of steroidogenesis by three ACTH concentrations, were not significantly different (Table 3, experiment I). However, combinations of submaximally effective concentrations of the effectors additively enhanced the stimulation of steroidogenesis by a low concentration of ACTH (Table 3, experiment II).

* $K_{0.5}$ value is the hormone concentration which elicits half the maximal steroidogenic response.

4. DISCUSSION

The lack of effect of adenosine and related compounds (except cyclic AMP) on basal corticosteroid production by isolated adrenocortical cells argues against a direct action of the nucleoside on adenylate cyclase activity.

The effect of both adenosine and theophylline on the stimulation of steroidogenesis by ACTH is qualitatively similar: both agents reduce the $K_{0.5}$ value for ACTH, although neither compound affects maximally stimulated steroid output. The latter observation suggests a site of action removed from the process of corticosteroid biosynthesis or release. Theophylline is known to enhance ACTH-induced steroid production by normal adrenal cells by its inhibition of cyclic AMP phosphodiesterase [17]. Adenosine has been reported to enhance hormonal stimulation of

Table 3. Experiment I. Effect of maximally effective concentrations on adenosine and theophylline on corticosteroidogenesis induced by sub-maximal concentrations of ACTH (5×10^{-5} IU/ml); Experiment II. Effect of sub-maximally effective concentrations of adenosine and theophylline on corticosteroidogenesis induced by sub-maximal concentrations of ACTH (5×10^{-5} IU/ml)

ACTH Concentration	Agent		μg Corticosterone/ rat/h ± S.E.M.	ACTH Concentration	Agent		μg Corticosterone/ rat/h + S.E.M.
	Adenosine	Theophylline			Adenosine	Theophylline	
	—	—	0.06 ± 0.01		—	—	0.06 ± 0.01
	0.2 mM	—	0.06 ± 0.01		0.02 mM	—	0.03 ± 0.01
0	—	2.0 mM	0.06 ± 0.01	0		0.2 mM	0.02 ± 0.00
	0.2 mM	2.0 mM	0.07 ± 0.01		0.02 mM	0.2 mM	0.03 ± 0.01
	—	—	0.75 ± 0.06		—	—	0.75 ± 0.03
	0.2 mM	—	0.87 ± 0.04		0.02 mM	—	0.78 ± 0.02
5×10^{-5} IU/ml	—	2.0 mM	0.96 ± 0.02	5×10^{-5} IU/ml	—	0.2 mM	0.78 ± 0.07
	0.2 mM	2.0 mM	0.98 ± 0.08		0.02 mM	0.2 mM	0.92 ± 0.02
	—	—	1.20 ± 0.05		—	—	1.11 ± 0.01
	0.2 mM	—	1.24 ± 0.03		0.02 mM	—	1.20 ± 0.07
1×10^{-3} IUml	—	2.0 mM	1.18 ± 0.11	1×10^{-3} IU/ml	—	0.20 mM	1.20 ± 0.09
	0.2 mM	2.0 mM	1.10 ± 0.10		0.02 mM	0.2 mM	1.23 ± 0.01
		Experiment 1				Experiment 2	

lipolysis through inhibition of phosphodiesterase [3]. Thus, by analogy it would appear that adenosine enhances ACTH-stimulated steroidogenesis by the same mechanism as theophylline. The additive effect of the two agents on ACTH-stimulated steroidogenesis supports this suggestion.

The lack of antagonism in the action of theophylline and adenosine noted above contrasts sharply with the mutually opposed actions of these compounds in adrenal tumour cells [15]. Future investigations may identify the differences between normal and tumour cells which lead to such divergent actions of adenosine on the two cell types, but until the issue is clarified the usefulness of using tumour cells to study the mode of action of ACTH is in doubt.

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