An Effect of Dexamethasone on Adenosine 3′,5′-Monophosphate Content and Adenosine 3′,5′-Monophosphate Phosphodiesterase Activity of Cultured Hepatoma Cells

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ABSTRACT The effect of dexamethasone on adenosine 3′,5′-monophosphate (cAMP) phosphodiesterase activity in cultured HTC hepatoma cells was investigated. Homogenates of these cells contain phosphodiesterase activity with two apparent Michaelis constants for cAMP (2–5 μM and 50 μM). At all substrate concentrations tested, phosphodiesterase activity was decreased 25–40% in cells incubated for 36 hr or more with 1 μM dexamethasone. Acid phosphatase activity in the same cells was not decreased. α-Methyl testosterone, 1 μM, was without effect on phosphodiesterase activity.

Incubation for 10 min with epinephrine plus theophylline increased the cAMP content of the HTC cells 3- to 6-fold. In cells incubated for 72 hr with dexamethasone, the basal concentration of cAMP was slightly increased and the increment produced by epinephrine plus theophylline was markedly increased. We believe that in many cells the so-called permissive effects of steroid hormones on cAMP mediated processes may be due to an effect of these hormones on cAMP phosphodiesterase activity similar to that observed in HTC cells incubated with dexamethasone.

INTRODUCTION

In several tissues, the effects of hormones that presumably elevate the intracellular concentration of cyclic adenosine 3′,5′-monophosphate (cAMP)[1] are enhanced by steroid hormones (1–3). We recently observed that the cAMP content of HTC hepatoma cells was increased several fold within 10 min after addition of epinephrine (or isoproterenol) and the increment was greater in cells that had been previously incubated with dexamethasone. This effect of dexamethasone and the so-called permissive effects of specific steroid hormones on cAMP mediated processes in other tissues could be secondary to a steroid-induced decrease in cAMP degradation. The homogeneous population of cultured cells appeared to offer an excellent system for investigation of the effects of a steroid hormone on cAMP phosphodiesterase activity. We found, as reported below, that phosphodiesterase activity was markedly decreased by incubation of HTC cells with dexamethasone. These observations are consistent with the view that the permissive effects of certain steroid hormones on processes regulated by cAMP may be the result of steroid-induced diminution in cAMP phosphodiesterase activity.

METHODS

HTC cells were maintained in monolayer cultures in 250 ml plastic Falcon flasks in Eagle's basal medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with Earle's salts, 10% fetal calf serum (Grand Island Biological Co.), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 36°C. Cells were detached from the flasks with trypsin, and $4–5 \times 10^5$ cells were added to Optilux Tissue Culture dishes (100×20 mm, Falcon Plastics, Div. of BioQuest, Oxnard, Calif.) with 9 ml of growth medium. After equilibration in a humidified atmosphere containing 5% CO_2, the dishes were incubated in an airtight box at 36°C. The medium was changed and dexamethasone was added to some dishes 2–3 days later. Thereafter medium was changed and fresh dexamethasone added every 2 days.

For measurement of cAMP, growth medium was removed and the cells were washed with serum-free medium. They were incubated for 1 hr, 36°C, in a humidified atmosphere with 5% CO_2, with 10 ml serum-free medium without dexamethasone before addition of epinephrine, theophylline,

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[1] *Abbreviations used in this paper:* cAMP, cyclic adenosine 3′,5′-monophosphate; dexamethasone, 9-fluoro-11β-17,21-trihydroxy-16α-methyl-pregma-1,4-diene-3,20-dione,21-phosphate; α-methyl-testosterone, 17-methyl-4-androsten-3-one; PGE_1, prostaglandin E_1.

TABLE I

Effect of Epinephrine and Theophylline on cAMP Content of HTC Cells

Exp. No.	Additions, concentration	cAMP in HTC cells: No theophylline	cAMP in HTC cells: Plus theophylline
		pmoles/mg protein	
1	None	0.7	0.9
	Epinephrine, 4 μM	0.7 (0.6, 0.8)	3.3 (3.0, 3.6)
2	None	—	0.8
	Epinephrine, 0.4 μM	—	2.3
	Epinephrine, 1.3 μM	—	3.7 (3.1, 4.3)
	Epinephrine, 2.7 μM	—	3.6 (3.3, 3.8)
3	None	0.8 (0.4, 1.1)	0.7 (0.4, 1.0)
	Epinephrine, 2.7 μM	—	4.8 (4.1, 5.5)
	Glucagon, 2 μg/ml	—	0.6 (0.3, 1.0)
4	None	—	0.4 (0.4, 0.4)
	isoproterenol, 2 μM	—	1.8±0.13*
	Prostaglandin E_1, 2.0 μg/ml	—	0.6 (0.5, 0.7)
	PGE_1 + isoproterenol	—	1.6 (1.6, 1.7)

Cells were incubated for 10 min with additions as indicated and with or without 1 mM theophylline. Data presented are the means of values from duplicate incubations with individual values in parentheses.
* Mean ±SEM, $n = 3$.

or other agents. 10 min later, incubations were terminated and cAMP was isolated and assayed, as previously described (5).

For measurement of cAMP phosphodiesterase activity, the medium was removed and the cells were rinsed twice with 10 ml of an ice-cold solution of 1 mM $MgSO_4$, 0.1 mM dithiothreitol, and 2 mM glyclyglycine, pH 7.4. They were scraped from the dish with a rubber policeman and homogenized in a tight-fitting glass Dounce homogenizer. Cell disruption was monitored by light microscopy. Samples of homogenate (75–100 μg protein) were incubated at 30°C in a total volume of 0.3 ml containing 25 μmoles of glycine buffer, pH 8.5, 8.3 μmoles $MgCl_2$, 2 pmoles cAMP-^{3}H (16.4 or 25.5 Ci/mmole) and unlabeled cAMP as indicated. The reaction was terminated by addition of 25 μmoles HCl, 88 nmoles cAMP, and 500 nmoles 5'AMP in 75 μl. After heating for 5 min at 70°C, 25 μmoles NaOH and 25 μmoles Tris, pH 8.0, in 50 μl was added, followed by 75 μl of 100 mM Tris buffer, pH 8, containing 0.18 mg *Crotalus adamanteus venom* (Sigma Chemical Co., St. Louis, Mo.). After incubation at 37°C for 30 min, adenosine-^{3}H was isolated for radioassay, as previously described (5). Under the conditions employed, the amount of cAMP hydrolyzed was proportional to time and to homogenate concentration.

Dexamethasone (phosphate salt) was kindly supplied by Dr. W. Gall of Merck & Co., Inc. (Rahway, N. J.), α-methyltestosterone by Dr. J. Handler, PGE_1 by Dr. J. Pike of the Upjohn Co. (Kalamazoo, Mich.), and crystalline glucagon by Eli Lilly & Co. (Indianapolis, Ind.). Solutions of L-epinephrine and L-isoproterenol (Sterling Winthrop Research Institute) were prepared from the bitartrate. cAMP-^{3}H was purchased from New England Nuclear Corp. (Boston, Mass.), and other nucleotides from Sigma.

RESULTS

After incubation for 10 min with 2.7 μM epinephrine or 2 μM isoproterenol and 1 mM theophylline, the cAMP content of HTC cells was increased severalfold (Table I). Epinephrine alone, theophylline alone, and glucagon or PGE_1 plus theophylline were without effect. In the presence of epinephrine and theophylline, cAMP levels remained elevated for at least 20 min. The effect of 2.7 μM epinephrine plus theophylline was prevented by 1 μM propranalol.

As shown in Table II, in cells grown with 10 μM dexamethasone for 72 or 48 hr, cAMP levels were somewhat higher than those of the respective control cells.[2] The increment produced by epinephrine was, however, considerably greater, and the phosphodiesterase activity was 30–40% lower in homogenates of cells that had been incubated with dexamethasone than it was in homogenates of control cells. After incubation of cells for 72 hr with 0.1, 1, or 10 μM dexamethasone, phosphodiesterase activity was decreased by about 40%. In cells incubated with 10 μM dexamethasone for 10 hr there was no significant change in phosphodiesterase activity, and the decrease at 24 hr was variable, but by 36 hr, the activity was decreased by approximately 25%.

Incubation of HTC cells with 1 μM α-methyltestosterone, which does not induce tyrosine aminotransferase (6) did not alter phosphodiesterase activity (Table III, experiments 1 and 2). In cells in which phosphodiesterase activity was decreased about 30% after incubation with 1 μM dexamethasone, acid phosphatase activity was not decreased and may have been slightly increased (Table III, experiments 2 and 3).

Homogenates of HTC cells contain phosphodiesterase with two apparent Michaelis constants for cAMP, one 2–5 μM and the other about 50 μM. These were not demonstrably altered by incubation with dexamethasone since the percentage decrease in activity was approximately the same at all substrate concentrations tested. Dexamethasone, 1 μM, present during assay of phosphodiesterase did not affect the activity of homogenates from control cells. Mixtures of homogenates from control and dexamethasone-treated cells exhibited diesterase activity equivalent to that expected on the basis of simple addition (Table III).

DISCUSSION

Although epinephrine in the presence of theophylline effectively caused accumulation of cAMP in HTC cells,

[2] It will be noted that the cAMP content of these control cells (with or without epinephrine) and that of the cells in experiment 3, Table I, and other experiments done at the same time, was considerably lower than that of the cells in experiments 1 and 2, Table I. The reasons for this are not clear. Cells from the same initial subculture of uncloned HTC cells were used for all of these experiments, but the experiments in which the higher cAMP values were observed were carried out several months earlier than the others. For the remainder of the studies reported here, HTC cells from another subculture were used.

TABLE II

Effect of Dexamethasone on cAMP Phosphodiesterase Activity and cAMP Response to Epinephrine

Exp. No.	Dexamethasone concentration	Time	cAMP Phosphodiesterase		cAMP in HTC cells	
			55 μM cAMP*	0.55 μM cAMP*	No epinephrine	Plus epinephrine
	μM	hr	nmoles/mg protein per 10 min		pmoles/mg protein	
1	0	72	4.2 (3.8, 4.5)‡	0.26 (0.25, 0.26)	0.4 (0.3, 0.4)	0.8 (0.8, 0.9)
	10	72	2.9 (2.9, 2.9)‡	0.16 (0.15, 0.18)	1.5 (1.5, 1.5)	3.2 (3.0, 3.3)
2	0	24	2.6 (2.5, 2.6)	0.26 (0.25, 0.27)		
	10	24	2.0 (2.0, 2.0)	0.21 (0.21, 0.21)		
	0	72	2.2 (2.1, 2.2)	0.21 (0.21, 0.21)	<0.2	1.5 (1.3, 1.7)
	0.1	72	1.3 (1.3, 1.3)	0.12 (0.12, 0.13)		
	1	72	1.2 (1.2, 1.2)	0.12 (0.12, 0.13)		
	10	48	1.3 (1.3, 1.3)	0.14 (0.12, 0.15)	0.3 (0.3, 0.3)	2.8 (2.8, 2.9)
3	0		3.4 (3.3, 3.4)	0.27 (0.27, 0.27)		
	10	10	3.6 (3.6, 3.6)	0.32 (0.31, 0.32)		
	10	24	3.5	0.29		
	10	36	2.6 (2.5, 2.7)	0.24 (0.24, 0.25)		
	1	36	2.7 (2.6, 2.8)	0.22 (0.22, 0.23)		

Data for phosphodiesterase activity are the means of values from duplicate incubations with the individual values in parentheses. Cyclic AMP content was determined on cells from other dishes after incubation for 10 min with 1 mM theophylline with or without 2.7 μM epinephrine. Data are as presented in Table I.

* Substrate concentration in assay.

‡ Assayed with 555 μM cAMP.

TABLE III

Effect of Dexamethasone and α-Methyltestosterone on cAMP Phosphodiesterase and Acid Phosphatase Activities

Exp. No.	Additions		Total hours*	cAMP phosphodiesterase		Acid phosphatase‡
	Steroid	Hours		55 μM cAMP	0.55 μM cAMP	
				nmoles/mg protein per 10 min		U
1	None		192	3.1 (3.1, 3.1)	0.22 (0.22, 0.22)	
	Dex	96	192	1.9 (1.9, 1.9)	0.14 (0.14, 0.14)	
	α-Met	96	192	3.3 (3.2, 3.4)	0.23 (0.22, 0.23)	
2	None		144	3.0 (2.8, 3.2)	0.22 (0.21, 0.22)	0.42 (0.42, 0.43)
	Dex	96	144	1.8 (1.7, 1.8)	0.15 (0.15, 0.15)	0.52 (0.51, 0.52)
	α-Met	96	144	3.0 (2.9, 3.0)	0.22 (0.22, 0.23)	
3	None		168	2.8 (2.8, 2.8)	0.20 (0.20, 0.20)	
	Dex	72	168	1.6 (1.5, 1.7)	0.11 (0.11, 0.11)	
	None		192	2.4 (2.4, 2.5)	0.22 (0.21, 0.22)	0.58 (0.55, 0.62)
	Dex	96	192	1.6 (1.5, 1.7)	0.16 (0.15, 0.17)	0.70 (0.69, 0.71)

Data for enzyme activities are reported as in Table II. Assays of a mixture of equal amounts of control and Dex homogenates from exp. 1 yielded activities of 2.4 and 2.5 nmoles/mg protein per 10 min (calculated, 2.6). With similarly mixed homogenates from exp. 2, the observed activities were 2.3 and 2.4 and the calculated, 2.4.

* Total time of incubation of cells after transfer to culture dishes. Dexamethasone (Dex), 1.0 μM, or α-methyltestosterone (α-Met), 1.0 μM, was present for the indicated number of hours at the end of this period.

‡ Acid phosphatase was assayed using *p*-nitrophenyl phosphate (Sigma) as substrate (7). 1 U equals 1 μmole of *p*-nitrophenol produced per 10 min/mg protein.

glucagon and PGE_1 failed to do so. Makman, on the other hand, found that lysates of HTC cells (also grown in stationary culture) contained adenylate cyclase activity that was stimulated by all three of these agents (8). The reasons for this apparent difference in the effects of glucagon and PGE_1 on intact and broken cells are unknown. It has been reported that adenylate cyclase activity in homogenates of a cultured adrenal tumor is enhanced by ACTH which has no effect on the cAMP content of the intact tissue (9).

It seems probable that the increased cAMP content of the HTC cells incubated with dexamethasone and the increased response to epinephrine are secondary to the diminished phosphodiesterase activity in these cells. It is tempting to speculate that in many types of cells the so-called permissive effects of steroid hormones on cAMP-mediated processes may be due to decreased phosphodiesterase activity. This could, for example, explain the effects of aldosterone on vasopressin-induced transport of water and electrolytes in toad bladder (1). In this tissue the elevation in cAMP concentration produced by vasopressin is increased dramatically by prior incubation with aldosterone (4). Dexamethasone also potentiates the effects of catecholamines and PGE_1 on accumulation of cAMP in cultured human fibroblasts (10). Decreased phosphodiesterase activity could also explain the reported effects of dexamethasone on hormone-stimulated lipolysis (2), although we have been unable to demonstrate any change in fat cell phosphodiesterase activity after incubation for 4 hr with this steroid. Fat cell phosphodiesterase activity was significantly decreased by treatment of rats with dexamethasone for 24–36 hr before sacrifice, but it would be incautious to conclude that this was a direct effect of the steroid.[3] It has been reported that phosphodiesterase activity is elevated in liver, kidney, skeletal muscle, and fat of adrenalectomized rats and can be restored to normal by treatment of the animals with L-methyl prednisolone (11). Such changes in phosphodiesterase activity could be a cause for the observed decrease in responsiveness of the rat liver to glucagon after adrenalectomy (12) and the effects of hydrocortisone on liver glycogen metabolism (3). The "permissive" effects of corticosteroids in perfused rat liver and heart have been extensively documented by Exton et al., who, however, have postulated a different mechanism of corticosteroid action (13, 14).

The relationship between the observed effects of dexamethasone on phosphodiesterase activity, and the reported effects on tyrosine aminotransferase induction in HTC cells (15) remains to be evaluated. There is at present no evidence that it is a causal one, since decreased phosphodiesterase activity was demonstrable only after 24 hr or more, whereas increased aminotransferase activity is evident within 3–4 hr (15). In many tissues, however, phosphodiesterase exists in more than one molecular form, perhaps differently localized intracellularly; thus early changes in the activity of a specific phosphodiesterase might go undetected when whole homogenates are assayed. In order to resolve this question and to elucidate the mechanism by which dexamethasone decreases phosphodiesterase activity in the HTC cells, a better understanding of the nature and regulation of phosphodiesterase(s) in these cells will be required. In view of information concerning the effects of steroid hormones on tyrosine aminotransferase induction in HTC cells (15), it seems probable that dexamethasone influences the synthesis of a protein that regulates the level of the phosphodiesterase itself or modifies its catalytic activity.

[3] Unpublished observations.

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