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Dexamethasone increases β-adrenoceptor density in human astrocytoma cells

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The density of β-adrenoceptors has been shown to vary under a number of experimental paradigms. Thus, during the process of catecholamine-induced desensitization, a reduction in the number of β-adrenoceptors occurs [1–5]. Conversely, drug-induced decreases in norepinephrine levels *in vivo* result in a compensatory increase in β-adrenoceptor density [6–8]. We have recently demonstrated [9] that the marked alterations in catecholamine-stimulated adenosine cyclic 3′,5′ monophosphate (cyclic AMP) accumulation which occur during growth of human astrocytoma cells in culture are mediated by similar changes in the density of β-adrenoceptors. Finally, β-adrenoceptor densities can be influenced by a number of chemicals of unrelated structure. Thus, sodium butyrate has been shown to induce β-adrenoceptors in Hela cells [10], while triiodothyronine increased the density of these receptors in rat heart [11] and in cultured heart cells [12].

Glucocorticoid steroids have been shown to be involved in the regulation of β-adrenoceptor density in the liver [13] and in the catecholamine responsiveness of lymphocytes [14, 15]. In this report we demonstrate that the synthetic glucocorticoid, 9α-fluoro-16α-methyl prednisolone (dexamethasone) can regulate the density of β-adrenoceptors and catecholamine-stimulated adenylate cyclase (EC 4.6.1.1) activity in human astrocytoma cells.

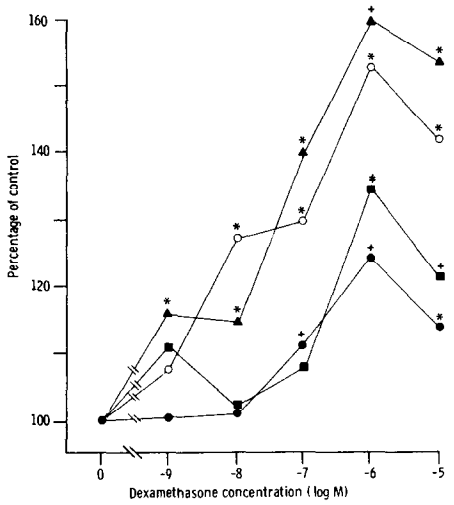

Fig. 1. Effect of dexamethasone concentration on basal, NaF- and isoproterenol-stimulated adenylate cyclase activity and the density of β-adrenoceptors. Adenylate cyclase activity and β-adrenoceptors were measured as previously described [9]. The concentration of ^{125}IHYP used in the β-adrenoceptor binding assay was 70 pM (80,000 dpm). The figure is constructed using the mean values obtained from the raw data where control values for adenylate cyclase activity were: basal (●), 6.3 ± 0.6; 10 mM NaF (■), 64.3 ± 1.4; 10 μM Isoproterenol (▲), 64.3 ± 1.3 pmoles min^{-1} mg^{-1} protein and for β-adrenoceptor binding (○) was 8.1 ± 0.2 fmoles mg^{-1} protein. Values are the means ± S.E.M. (N = 5). Values are significantly different from controls as indicated: $P < 0.001$ (*); $P < 0.01$ (†); $P < 0.02$ (‡); N = 5.

The human astrocytoma cells (1321N1) used in these experiments have been extensively studied with respect to their hormone-sensitive cyclic AMP system [5, 9, 16, 17]. The cells were cultured in Dulbecco's modified Eagle's medium as previously described [9]. For experimental purposes the cells were grown in 150 mm plastic tissue culture dishes (Falcon) for 8 days with medium changed at 3-day intervals. After incubation for 6 hr with the indicated concentrations of dexamethasone, the growth medium was aspirated, and the cell sheets were rinsed twice with an ice-cold lysing medium consisting of 1 mM Tris–HCl, pH 7.8, at 0°; 1 mM $MgCl_2$. The cells were allowed to swell for 15 min and were then lysed by scraping the culture dish surface with a rubber policeman. The lysate was diluted with ice-cold 50 mM Tris–HCl, pH 7.8, and centrifuged at 17,000 *g* for 10 min at 4°. The pellet was resuspended in buffer and assayed immediately. Adenylate cyclase activity was measured by the method of Salomon *et al.* [18] as we have previously described [9]. ^{125}I-iodohydroxybenzylpindolol was prepared [19] and the density of β-adrenoceptors assessed as has been reported for 1321N1 cells [9]. Protein concentration was determined by the method of Lowry *et al.* [20].

Figure 1 demonstrates that dexamethasone produced a dose-dependent increase in isoproterenol-stimulated adenylate cyclase activity which correlated with a similar increase in the number of β-adrenoceptors. This increase in β-adrenoceptor number and isoproterenol-stimulated adenylate cyclase activity was 150–160 per cent of control values. At higher dexamethasone concentrations, basal and NaF-stimulated adenylate cyclase activities were also significantly elevated above that of control. In addition, we have observed that adenylate cyclase activity in the presence of 5′-guanylyl-imidodiphosphate is increased in membranes from dexamethasone treated cells.*

The glucocorticoid dependent increase in isoproterenol-stimulated adenylate cyclase activity was a time-dependent process in which β-adrenoceptor number and isoproterenol-stimulated adenylate cyclase activity increased in parallel. In cells incubated with 1 μM dexamethasone, a lag of approximately 60 min occurred, after which time the density of receptors and isoproterenol-stimulated enzyme activity increased to a maximal value (60 per cent above control) after 6–8 hr and remained constant for at least 24 hr in the presence of dexamethasone. As shown in Fig. 2, the dexamethasone-induced increase in ^{125}I-hydroxybenzylpindolol (^{125}IHYP) binding was due to an increase in the density of β-adrenoceptors per cell rather than to an alteration in the affinity of radioligand. Furthermore, the capacity of isoproterenol to inhibit ^{125}IHYP binding was unaffected by incubation of cells with dexamethasone.*

It was of interest to characterize further the nature of these steroid-induced changes in β-adrenoceptor density. For example, does the enhancement of receptor density

* Data not shown.

Table 1. Inhibition of dexamethasone-induced β-adrenoceptor number by cycloheximide*

Incubation time with drugs (hr)	No drug	Cycloheximide	Dexamethasone	Cycloheximide + Dexamethasone
6	—	8.2 ± 0.1	19.5 ± 1.6	10.0 ± 0.8
24	12.7 ± 0.8	11.9 ± 0.7	19.7 ± 0.7	10.5 ± 0.6

* Cells were grown in 100 mm dishes for four days, then incubated with cycloheximide (5μg/ml) and/or dexamethasone (1 μM) for the times indicated. The cells were then lysed and the density of β-adrenoceptors was determined. Data are presented as fmoles mg^{-1} protein and are the means of five individual dishes ± S.E.M. These data are representative of three similar experiments.

require protein synthesis or are latent receptors expressed as a result of incubation of cells with the steroid? As demonstrated in Table 1, the appearance of the β-adrenoceptor is dependent on protein synthesis since cycloheximide (5μg ml^{-1}), at a concentration which inhibited [^{3}H]leucine incorporation into protein by greater than 90 per cent, inhibited the increase in β-adrenoceptor density induced by dexamethasone. Although these data do not directly demonstrate the synthesis of new receptor protein, they are consistent with the idea that dexamethasone affects the synthesis of protein which ultimately results in an increase in the specific activity of β-adrenoceptors in these cells.

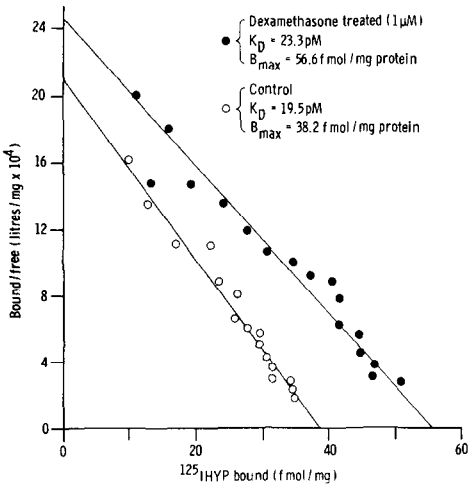

Fig. 2. Scatchard analysis of ^{125}IHYP binding to crude membranes prepared from control or dexamethasone-treated 1321N1 cells. Approximately 35 μg of cell lysate protein were incubated with various concentrations (30–400 pM) of ^{125}IHYP and the amount of radioligand specifically bound at each concentration was determined. The ratio of the amount of bound ligand to free ligand (ordinate) is plotted vs the amount of ^{125}IHYP bound mg^{-1} of protein [22]. Data points are the means of triplicate determinations. Lines represent the best least squares fit. The intercepts of the abscissa represent maximal binding capacity and the slope is equal to $-1/K_D$. The data shown is representative of three separate experiments, the mean K_D values of which were: control, 21.9 ± 1.4 pM; 1 μM dexamethasone-treated cells, 24.5 ± 2.6 pM.

The data reported here suggests that dexamethasone can increase the concentration of the components of the adenylate cyclase system in the plasma membrane of 1321N1 astrocytoma cells. Brostrom *et al.* [21], using C6 glioma cells, have also observed an increase in basal and both NaF- and norepinephrine-stimulated adenylate cyclase activities in homogenates prepared from glucocorticoid-treated cells. These workers attributed these changes to an increase in the amount of the catalytic component of adenylate cyclase. It is of interest to point out that while dexamethasone causes an increase in the expression of various components of the β-adrenoceptor/adenylate cyclase system of astrocytoma cells, lymphocytes [14, 15] and C-6 glioma cells [21], treatment of adrenalectomized rats with glucocorticoids results in a decrease in β-adrenoceptors and catecholamine-stimulated adenylate cyclase activity in liver membranes [13]. The physiological significance of these seemingly opposite effects is unknown.

The time course of the effect of dexamethasone on the density of β-adrenoceptors and the dependence on protein synthesis suggest that the synthesis of β-adrenoceptors may be enhanced in response to glucocorticoids. Such an alteration in receptor number could also explain the increased response of lymphocyte and leukocyte cell populations to catecholamines in cells taken from control and high-dose corticosteroid therapy patients [14] and *in vitro* experiments [15] and, as such, may have clinical significance.

In summary, we conclude that growth of human astrocytoma cells in the presence of dexamethasone results in a concentration-dependent increase in the number of β-adrenoceptors per cell which correlates with an increase in catecholamine-stimulated adenylate cyclase activity. Experiments with cycloheximide indicate that the increase in β-adrenoceptor density is dependent on protein synthesis. Furthermore, this dexamethasone-induced increase in β-adrenoceptor density may potentially provide a model system in which β-adrenoceptor turnover can be investigated.

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Further studies on the inhibition of Leydig cell testosterone production by cannabinoids

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Earlier reports from our laboratory have shown that Δ^1-tetrahydrocannabinol (Δ^1-THC), the psychoactive principle of marihuana, can inhibit the synthesis of testosterone by isolated Leydig cells [1, 2] and of progesterone by isolated luteal cells [3]. A detailed study of this effect revealed that the site of inhibition was the cholesterol esterase which provides precursor for steroid hormone biosynthesis [4]. A number of substances that are structurally related to Δ^1-THC and that do not exhibit psychoactivity occur in cannabis preparations to which subjects would be exposed when using marihuana, hashish, etc. [5]. Our previous reports [1–3] suggested that the non-psychoactive cannabinoids may be physiologically active, since one of these, namely cannabinol (CBN), showed a potency similar to that of Δ^1-THC in lowering testosterone levels. We now present the results of a more extensive study that included the major naturally occurring cannabinoids (Fig. 1).

The Leydig cells were prepared from testes of 60- to 90-day-old mice (Charles River, CD-1) as described previously [2]. Aliquots containing 2×10^6 cells each in Krebs–Ringer bicarbonate buffer (2 ml, pH 7.4) were then incubated for 2 hr with 25 mIU hCG (Organon "Pregnyl") at 32° under 95%/O_2 : 5% CO_2. Testosterone production averaged 20.3 ng/10^6 cells for non-drug-treated controls. The can-

Table 1. Inhibition of testosterone production in isolated mouse Leydig cells*

	Inhibition of testosterone production						
	Cannabinoid concentration (μM)						
	0.032	0.16	0.32	1.6	3.2	9.0	16
CBG	0.5 ± 0.01	15.6 ± 0.18†	25.3 ± 1.6‡	47.7 ± 0.87§			
CBD			16.1 ± 0.75‡	62.1 ± 2.83§	74.5 ± 2.88§	82.9 ± 2.59§	85.4 ± 1.69§
CBCy	3.0 ± 0.06	9.2 ± 0.32†	22.6 ± 0.53§	53.5 ± 1.28§			
CBN		5.5 ± 0.25	19.1 ± 0.31§	51.3 ± 1.25§	68.8 ± 1.62§		
Δ^1-THC			35.4 ± 3.5†	36.7 ± 0.71†	49.6 ± 4.0‡	65.4 ± 12.4§	84.5 ± 30.0§
CBC			17.5 ± 0.24§	13.7 ± 0.46‡	25.5 ± 0.74§	46.4 ± 2.8§	63.7 ± 1.13§
Olivetol	4.6 ± 0.06	1.5 ± 0.05	3.3 ± 0.10	15.4 ± 0.28§			

* Values are per cent inhibition ± S.E. (N = 5). Individual controls were provided for each cannabinoid. Abbreviations: CBG, cannabigerol; CBD, cannabidiol; CBCy, cannabicyclol; CBN, cannabinol; Δ^1-THC, Δ^1-tetrahydrocannabinol; and CBC, cannabichromene.
† $P < 0.05$.
‡ $P < 0.005$.
§ $P < 0.001$.