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PARADOXICAL EFFECTS OF GLUCOCORTICOIDS ON REGULATION OF PLASMINOGEN ACTIVATOR ACTIVITY

MEDIATION BY GLUCOCORTICOID RECEPTORS

PATRICIA A. BAROUSKI-MILLER* and THOMAS D. GELEHRTER†
Departments of Human Genetics and Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A.

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Summary—Dexamethasone, a synthetic glucocorticoid, decreases the plasminogen activator (PA) activity of HTC rat hepatoma cells in tissue culture. Paradoxically, dexamethasone enhances the cyclic nucleotide stimulation of PA activity in these cells 2–4-fold. In this report, we investigated whether this paradoxical glucocorticoid effect is mediated by the same proximal events which mediate such direct regulatory actions of glucocorticoids as the induction of tyrosine aminotransferase activity. We compared the concentration-dependences for several classes of steroids, previously classified as full agonists, partial agonists, antagonists or inactive steroids with respect to induction of the transaminase, for both enhancement of cyclic nucleotide stimulation of PA activity and induction of tyrosine aminotransferase activity in parallel cultures. The full agonists dexamethasone and cortisol, the partial agonists deoxycorticosterone and 11β-hydroxyprogesterone, the inactive steroid tetrahydrocortisol, and the antagonist 17α-methyltestosterone exhibited similar potencies with respect to both phenomena. Furthermore, when cells were incubated with both dexamethasone and 17α-methyltestosterone, the latter blocked enhancement by dexamethasone in a concentration-dependent fashion. We conclude that glucocorticoid enhancement of cyclic nucleotide stimulation of PA activity is mediated by the same glucocorticoid receptors which mediate direct regulatory effects.

INTRODUCTION

Dexamethasone, a synthetic glucocorticoid, dramatically decreases the plasminogen activator (PA) activity of rat hepatoma tissue culture (HTC) cells through the induction of a specific inhibitor of PA [1, 2]. In contrast, cyclic nucleotides stimulate both the cell-associated and extracellular activity of this serine protease. Paradoxically, dexamethasone enhances this stimulation 2–4-fold [3]. This latter glucocorticoid effect can be dissociated from the direct glucocorticoid regulatory action on PA: enhancement of cyclic nucleotide stimulation by dexamethasone is also observed in variant HTC cells which are selectively resistant to glucocorticoid inhibition of PA activity [4].

Current models of steroid hormone action propose that steroids diffuse freely into cells and bind reversibly to specific cytoplasmic receptors. Following activation to a chromatin-binding form, the receptor–steroid complexes translocate to the nucleus, and are thought to bind to specific sites on chromatin and mediate the metabolic effects of the steroid by transcriptional regulation of specific genes [5, 6]. Evidence for this model includes the direct correlations which have been reported between cytoplasmic binding, nuclear binding, and induction of tyrosine aminotransferase (TAT) by several different classes of steroids in HTC cells [7, 8]. If glucocorticoid enhancement of cyclic nucleotide stimulation of PA activity in HTC cells is mediated by the same proximal events that mediate the direct regulatory effects of glucocorticoids (e.g. induction of TAT), the dose–response relationships for various steroids should be comparable. In other systems, however, the concentrations of dexamethasone required to elicit permissive and interactive effects of glucocorticoids have varied widely [9–11] and the mechanisms of such interactions of glucocorticoids with other hormones and effectors remain to be identified [9, 12].

In this study, we measured the dose–response relationships of several classes of steroids for enhancement of cyclic nucleotide stimulation of PA activity and for induction of TAT activity in parallel cultures at 24 h, a time when both responses should be stable and maximal. Samuels and Tomkins [13] have previously classified steroids on the basis of their ability to induce TAT. Full agonists maximally induce the biological response; partial agonists, which

*Present address: Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20205, U.S.A.
†To whom correspondence should be addressed: Thomas D. Gelehrter, MD, Department of Human Genetics Box 015, University of Michigan Medical School, Ann Arbor, MI, 48109 U.S.A.

also bind to the glucocorticoid receptor, induce the response suboptimally even at high concentrations; antagonists bind to the receptor, but fail to elicit a biological response; and, inactive steroids neither bind to the receptor nor elicit a response. We report here that these steroids exhibit the same relative potencies in the induction of TAT activity and enhancement of cyclic nucleotide stimulation of PA activity. Furthermore, the antagonist 17α-methyltestosterone blocks the enhancement by the agonist dexamethasone in a concentration-dependent manner. We conclude that the enhancement phenomenon is mediated by the same glucocorticoid receptors which mediate the direct actions of glucocorticoids.

MATERIALS AND METHODS

Materials

Eagle's Minimal Essential Medium, calf and fetal bovine sera, glutamine, trypsin and neomycin were obtained from GIBCO Laboratories, Grand Island, New York. Dexamethasone (9α-fluoro-16α-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione) was a gift from Dr Walter Gall of Merck & Co. Cortisol (11β,17α,21-trihydroxy-4-pregnene-3,20-dione), deoxycorticosterone(21-hydroxy-4-pregnene-3,20-dione), 11β-hydroxyprogesterone (11β-hydroxy-4-pregnene-3,20-dione), tetrahydrocortisol (3α, 11β, 17α, 21-tetrahydroxy-5β-pregnan-20-one), and 17α-methyltestosterone (17α-methyl-17β-hydroxy-4-androsten-3-one) were purchased from Steraloids Inc., Wilton, New Hampshire and were each reported to migrate as a single spot on thin layer chromatography. Fibrinogen (98% clottable), 8-bromadenosine-3′:5′-cyclic monophosphoric acid and methylisobutylxanthine (MIBX) were obtained from Calbiochem–Boehring, San Diego, California. ^{125}I-labeled fibrinogen (sp. act. ~ 150 mCi/g protein) was purchased from Amersham, Arlington Heights, Illinois. Plasminogen was purified from outdated human plasma by affinity chromatography [14]. All other compounds were of at least reagent grade.

Cell culture

HTC cells were grown in monolayer culture without antibiotics in Eagle's Minimal Essential Medium supplemented with 50 mM tricine (*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine), 0.5 g/l sodium bicarbonate, 2 mM glutamine, 0.02% calcium chloride, 5% calf serum and 5% fetal bovine serum.

Enzyme assays

Tyrosine aminotransferase (L-tyrosine: 2-oxoglutarate aminotransferase, EC 2.6.1.5) activity was measured as previously described by Spencer and Gelehrter [15]. Plasminogen activator activity in cell extracts (in 0.2% Triton X-100) and in medium conditioned by the cells was determined by the plasminogen-dependent solubilization of ^{125}I-labeled fibrin coated on multiwell plates as described elsewhere [1, 16]. Solubilized radioactivity was corrected for background release and then expressed as a percentage of the total radioactivity solubilized by 50 μg trypsin. The various steroids, 8-Br-cAMP, MIBX, ethanol, and dimethyl sulfoxide did not alter either fibrinolytic activity or background release when added directly to the assay.

Glucocorticoid treatment of cells

Cells were plated at 1 to 2 × 10^6 cells/35 mm tissue culture dish in serum-supplemented medium. After the cells had grown to 80–90% confluence, the monolayers were washed twice with serum-free medium and then incubated for 24 h at 37°C in serum-free medium containing 0.1% bovine serum albumin, 0.02% calcium chloride, 50 μg/ml neomycin and 8-Br-cAMP, MIBX and various steroids at the concentrations described in the Figure legends. Maximal stimulation of PA activity by cyclic nucleotides in HTC cells is observed when cells are incubated with the cAMP derivative 8-Br-cAMP and the potent phosphodiesterase inhibitor MIBX [3]. The maximal concentrations of ethanol (1.1%) and dimethyl sulfoxide (0.5%) used did not affect either the basal or induced levels of TAT or PA activity.

RESULTS

Concentration dependence of dexamethasone induction of TAT activity, inhibition of PA activity, and enhancement of 8-Br-cAMP stimulation of PA activity.

Initially, we examined the dose–response relationships for dexamethasone induction of TAT activity, inhibition of PA activity, and enhancement of cyclic nucleotide stimulation of PA activity. Inhibition of PA activity and induction of TAT activity were measured in the same cultures, as described in Materials and Methods; the enhancement phenomenon was examined in parallel cultures which contained 8-Br-cAMP and MIBX as well as dexamethasone. In this experiment, the concentration-dependence upon dexamethasone is virtually identical for all three phenomena (Fig. 1). Half-maximal response was obtained at 4 nM dexamethasone, and maximal response at approx 20 nM.

Steroid specificity of the induction of TAT activity and enhancement of cyclic nucleotide stimulation of PA activity

We measured the concentration-dependence of steroidal induction of TAT activity and enhancement of cyclic nucleotide stimulation of PA activity in parallel cultures after 24 h of incubation, a time when both effects should be stable and maximal (Fig. 2). As previously reported by Samuels and Tomkins [13] and by Gelehrter and McDonald [17], dexamethasone and cortisol maximally induced TAT activity. Half-maximal induction was observed at 4 and 50 nM, respectively. Deoxycorticosterone and

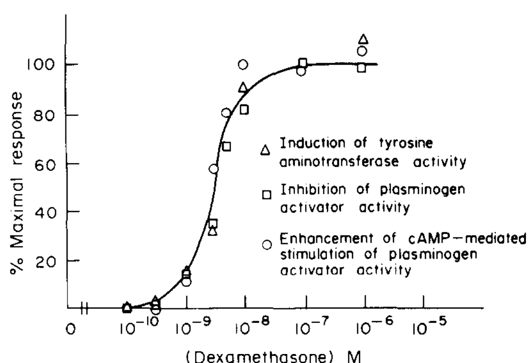

Fig. 1. Concentration-dependence of dexamethasone induction of TAT activity, inhibition of PA activity, and enhancement of 8-Br-cAMP stimulation of PA activity. Parallel cultures of HTC cells were incubated for 24 h in serum-free medium with increasing concentrations of dexamethasone in the absence (△ induction of TAT activity: □ inhibition of PA activity) or presence (○ enhancement of 8-Br-cAMP stimulation of PA activity) of 3 mM 8-Br-cAMP and 1 mM MIBX. Ethanol concentration was 0.2%, and dimethyl sulfoxide concentration 0.5%. PA activity and TAT activity were measured as previously described; results are expressed as a percentage of the maximal response. Maximal induction was 5-fold, maximal inhibition was 87%, and maximal enhancement was 2-fold.

11β-hydroxyprogesterone submaximally induced TAT activity. Deoxycorticosterone induced TAT to slightly higher levels than did 11β-hydroxyprogesterone and was effective at lower concentrations. Neither the antagonist, 17α-methyltestosterone, nor the inactive steroid, tetrahydrocortisol, induced TAT activity.

Similar, although not identical, concentration-dependences were obtained for the enhancement phenomenon (Fig. 2B). The full agonists, dexamethasone and cortisol, elicited maximal enhancement at the same relative potencies observed for the induction of TAT activity. Deoxycorticosterone and 11β-hydroxyprogesterone again behaved as partial agonists, enhancing cyclic nucleotide stimulation of PA activity to 50 to 60% of the maximal response. However, the concentrations required for half-maximal enhancement by both full and partial agonists were somewhat lower than those required for half-maximal induction of TAT. Neither the antagonist nor the inactive steroid caused any enhancement of cyclic nucleotide stimulation.

Competitive interactions between steroids

Antagonists block the induction of TAT and the inhibition of amino acid transport by full agonists, presumably by competing for binding to the glucocorticoid receptor [13, 17]. If enhancement is indeed mediated by glucocorticoid receptors, then the addition of 17α-methyltestosterone or progesterone should similarly block dexamethasone enhancement of stimulation of PA activity by cAMP derivatives. As illustrated in Fig. 3, 17α-methyltestosterone does block the enhancement response elicited by dexamethasone in a concentration-dependent fashion. In the absence of dexamethasone, these concentrations of 17α-methyltestosterone have no effect on PA activity. Progesterone similarly blocks dexamethasone enhancement; However, at high concentrations ($\geqslant 10\ \mu M$) in the absence of dexamethasone, progesterone itself enhances cAMP effects (data not shown). Similar anomalous effects of high concentrations of progesterone were observed on inhibition of amino acid transport in HTC cells [17].

DISCUSSION

The concentration-dependence of the dexamethasone induction of tyrosine aminotransferase activity is directly correlated with the binding of

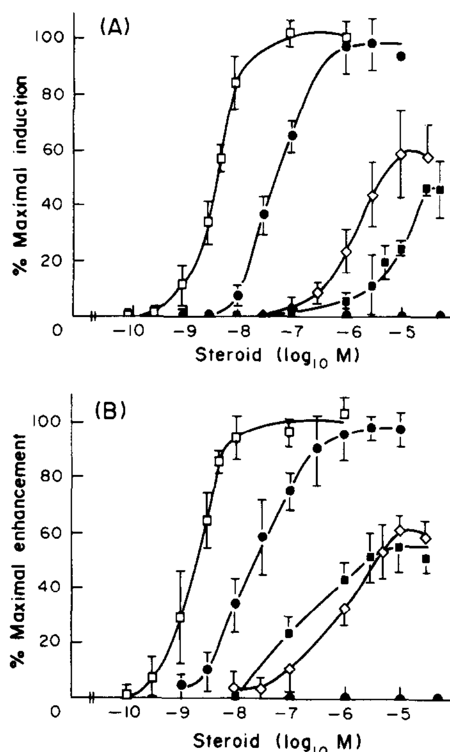

Fig. 2. Steroid specificity of the induction of TAT activity and of the enhancement of 8-Br-cAMP stimulation of PA activity. HTC cells were incubated for 24 h in serum-free medium in the absence (TAT samples) or presence (PA samples) of 3 mM 8-Br-cAMP and 1 mM MIBX with increasing concentrations of: (□) dexamethasone, (●) cortisol, (◇) deoxycorticosterone, (■) 11β-hydroxyprogesterone, (◆) 17α-methyltestosterone, or (○) tetrahydrocortisol; TAT activity (Fig. 2A) and both cell-associated and extracellular PA activity (Fig. 2B) were measured in duplicate assays of single (TAT) or duplicate (PA) cultures. Results are expressed as the percentage (mean ± SEM) of the maximal response observed in dexamethasone-treated cultures. Data are derived from at least three experiments at each concentration for all the steroids tested except 17α-methyltestosterone and tetrahydrocortisol; those data are from two experiments. Maximal induction of TAT was 8.8 ± 0.2-fold (mean + SEM) and maximal enhancement of PA was 3.9 ± 1.0-fold.

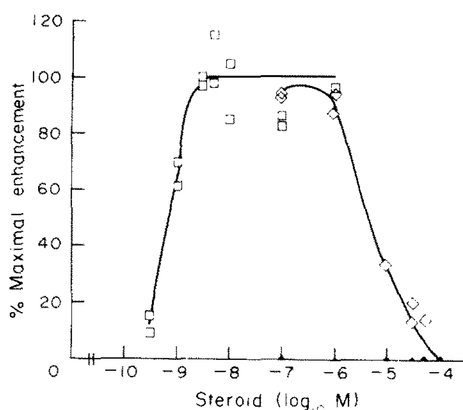

Fig. 3. Enhancement of 8-Br-cAMP stimulation of PA activity by dexamethasone: competition by 17α-methyltestosterone. HTC cells were incubated in serum-free medium containing 1 mM 8-Br-cAMP and 1 mM MIBX with increasing concentrations of: (□) dexamethasone; (♦) 17α-methyltestosterone; or, (○) 17α-methyltestosterone and a constant concentration (5 nM) of dexamethasone. Each point is derived from duplicate assays of a single culture and is expressed as a percentage of the maximal enhancement observed in dexamethasone-treated cultures. Maximal enhancement was 2-8-fold.

dexamethasone to cytoplasmic receptors, and with the nuclear binding of receptor–steroid complexes in HTC cells [5–8]. Furthermore, the concentration-dependences for the dexamethasone induction of tyrosine aminotransferase, alkaline phosphodiesterase I and cell adhesiveness, and the inhibition of amino acid transport and plasminogen activator activity are virtually identical (Fig. 1 and Ref. 5–8, 17–21) consistent with these phenomena all being mediated by the same glucocorticoid receptors. Additional support for this view comes from studies of the steroid specificity of these effects using various classes of steroids including full agonists, partial agonists, antagonists and inactive steroids [13, 17, 21]. Similar observations have been made for direct glucocorticoid effects in other experimental systems [22].

In contrast, the dose–response relationships for permissive or interactive effects of glucocorticoids with other hormones and effectors vary considerably. The concentration-dependence of the permissive effect of dexamethasone on dibutyryl cAMP induction of TAT in HTC cells is right-shifted relative to the concentration-dependence for direct glucocorticoid induction of TAT in these cells [9]. However, glucocorticoid inhibition of vasoactive intestinal peptide stimulation of cAMP accumulation and prolactin secretion in cultured rat pituitary cells occurs at picomolar concentrations and is glucocorticoid-specific [11].

In HTC cells, dexamethasone both inhibits PA activity and, paradoxically, enhances the stimulation of PA activity by cyclic nucleotides. In this report, we have presented two lines of evidence that this paradoxical steroidal effect on cyclic nucleotide regulation of PA activity is mediated by glucocorticoid receptors. First, several classes of steroids exhibit similar potencies with respect to both induction of TAT and enhancement of cyclic nucleotide stimulation of PA. Second, the antagonist 17α-methyltestosterone blocks the enhancement by dexamethasone in a concentration-dependent manner.

Although our data suggest that the paradoxical effects of dexamethasone on PA activity are mediated by glucocorticoid receptors, it should be noted that the enhancement of cyclic nucleotide stimulation of PA activity occurs at slightly lower concentrations of dexamethasone than does the induction of transaminase activity (Figs 2 and 3). A similar leftward shift in concentration-dependence is also noted for cortisol and for the partial agonists deoxycorticosterone and 11β-hydroxyprogesterone (Fig. 2). Mercier *et al.*[23] have reported a similar, though even more striking, leftward shift in the concentration-dependence for a dexamethasone-induced biological effect relative to binding of dexamethasone to glucocorticoid receptors. In FU5-5 rat hepatoma cells, the dose–response curve for dexamethasone induction of tyrosine aminotransferase is shifted to the left approx. 7-fold relative to that for dexamethasone binding to whole cells, and to that for induction of glutamine synthetase. The explanation for such discrepant dose–response relationships is not known, but the occurrence of such non-linear relationships between the concentration-dependence for glucocorticoid binding and biological action suggests greater complexity than that included in current models of glucocorticoid action [5, 6, 8, 22].

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