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Glucocorticoid enhancement of glucocorticoid production by cultured ovine adrenocortical cells

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The present study examines the effect of chronic treatment with glucocorticoids on the steroidogenic activity of ovine adrenocortical cells in vitro. Cells cultured in the presence of 10^{-9} to 10^{-5} M dexamethasone produced more glucocorticosteroids in response to $ACTH_{1-24}$, forskolin or 8 BrcAMP than did control cells. Such an enhancing effect required more than 5 h of treatment and was maximal at 30 h; it was both concentration-dependent and steroid-specific. The maximal secretion of corticosteroids was observed when cells were exposed to 10^{-7} M dexamethasone; with higher concentrations the response to $ACTH_{1-24}$ decreased steadily; the ED_{50} was 2.8 ± 0.8 nM. Cortisol and corticosterone enhanced $ACTH_{1-24}$-induced steroidogenesis to the same extent as dexamethasone, but at concentrations roughly 100-fold higher than for dexamethasone. Testosterone and 17β-oestradiol had no enhancing effect. Dexamethasone not only enhanced the maximal steroidogenic response to $ACTH_{1-24}$ but also decreased its ED_{50} 3-fold. Treatment of cultures with the antiglucocorticoid RU 38486 resulted in a dose-dependent, time-dependent, decrease in $ACTH_{1-24}$-induced corticosteroid output. Moreover, RU 38486 antagonized the enhancing effect of dexamethasone. The production of corticosteroids by dexamethasone-treated cells incubated in the presence of 22(*R*)-hydroxycholesterol or of exogenous pregnenolone was similar to that of control cells. The enhancing effect of dexamethasone was also observed when cultures were performed in the absence of insulin and/or in serum-free media. These data suggest that chronic exposure to glucocorticoids is necessary for the full steroidogenic activity of ovine adrenocortical cells. Moreover, they indicate that glucocorticoids exert their effect at least at two different levels in the cell: (i) on the adenylate cyclase system and (ii) at step(s) beyond cAMP but before pregnenolone formation.

Introduction

Many studies suggest that adrenal steroid synthesis is, in part, modulated by steroids synthesized within the adrenal cortex as end products or intermediates [1–11]. Most of these studies have shown that acute and/or chronic exposure of

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Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; 8-BrcAMP, 8 bromo-cyclic adenosine monophosphate.

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adrenal cells to exogenous corticosteroids (usually in concentrations in the micromolar range and often higher) decreases ACTH-induced steroidogenesis [1–4,6–8,10,11]. However, the results of other works are not in agreement with these findings [12,13] and some data might even suggest a positive effect of glucocorticoids on the steroid output of adrenocortical cells [5,9]. The mechanism(s) by which high concentrations of glucocorticoids can affect adrenocortical function is (are) not well understood. It has been proposed that glucocorticoids may decrease the binding of ACTH to its receptor [14] and/or inhibit ACTH-induced intra-cellular cAMP production [9]. However, a direct interference with ACTH receptors has been disputed [7] and even an enhancement of the ACTH-stimulated cAMP production of adrenal membranes by glucocorticoids has been reported [15]. From other studies, it would appear that exogenous steroids might modulate steroid hydroxylase activity by direct steroid-enzyme interactions [7,16]. On the other hand, adrenocortical cells possess high affinity glucocorticoid receptors [11,17,18]. This suggest that low concentrations of glucocorticoids could also regulate some aspects of adrenocortical activity. In a recent work we have observed that adrenocortical cells from adult sheep cultured for 24 or 48 h in the presence of dexamethasone produce more cAMP in response to ACTH than do control cells, and that the ED_{50} of dexamethasone for this effect is in very good agreement with the apparent dissociation constant reported for the glucocorticoid receptor of the adrenal cortex [19].

In the present study, we have examined whether exposure to glucocorticoids could also affect the output of corticosteroids by cultured ovine adrenocortical cells. The data indicate that glucocorticoids do indeed modulate in a biphasic manner adrenocortical steroidogenesis, and that this effect involves together with an enhanced activity of the $ACTH_{1-24}$-sensitive adenylate cyclase system, some steps located beyond cAMP.

Materials and Methods

Adrenocortical cells isolation and culture

Ovine adrenal glands were obtained from a local slaughter house and transported to the laboratory in ice-cold physiological saline. Isolated adrenocortical cells were prepared and cultured at a density of about 150000 cells/cm^2 as previously described [20], except that in some cases insulin (10 μg/ml) and/or horse serum (2%) were omitted from the culture medium. Treatment of cells with steroids or inhibitors started at least 24 h after seeding (day 1). The medium was renewed daily. At the end of the experiments, cells were detached from the culture dishes in 0.9% NaCl, 10 mM Hepes, 1 mM EDTA (pH 7.4) containing 1 mg/ml trypsin and counted in a Coulter Counter.

Incubation procedure

At selected times during the culture, the medium was aspirated and replaced with 1 ml fresh medium containing 0.5 mM 1-methyl-3-isobutylxanthine and various concentrations of $ACTH_{1-24}$, or 8-bromo-cyclic adenosine monophosphate (8-BrcAMP) or forskolin and in the absence or presence of steroids and/or inhibitors. Culture dishes were then placed in an incubator at 37°C under an atmosphere of 95% air/5% CO_2 for a variable length of time. At the end of the incubation period, aliquots of the medium were withdrawn and added to tubes containing 3 ml absolute ethanol precooled at −20°C for cAMP determination. The remaining medium was frozen until assayed for steroid contents.

Assays for cAMP and corticosteroids

Measurement of cAMP content was performed as described [20]. Corticosteroids released by cells incubated in the presence of dexamethasone were measured by a radiocompetition assay using plasma from female rats adrenalectomized 10 days prior to death (as a source of transcortin) and both labelled and unlabelled corticosterone [21]. Since dexamethasone cross reacted less than 0.004%, no correction was performed. Results were expressed as 'corticosteroids'. For these cells cultured in the presence of cortisol, corticosterone released was measured by radioimmunoassay using an antiserum (gift from Dr. A. Kowarski) cross-reacting 100% with corticosterone, 61% with 11-deoxycorticosterone, 35% with progesterone and 1% with cortisol. For cells exposed to corticosterone, cortisol released was measured using an antibody (kindly provided by N. Poulin) cross-reacting 100%

with cortisol and 70% with cortisone. Other C21 steroids cross-reacted less than 1%. In both of these latter cases, results were corrected for the cross-reactivity of exogenous cortisol and corticosterone in the assays of corticosterone and cortisol, respectively. All cAMP and steroid determinations were carried out in triplicate.

Data analysis

Selected data are presented herein with their S.E.M.; they were obtained in each case from three different wells. Paired Student's *t*-test was used throughout for statistical analysis. Every experiment was reproduced at least three times with similar results.

Materials

$ACTH_{1-24}$ (synacthen) was obtained from Ciba (Rueil-Malmaison, France); forskolin was from Calbiochem (La Jolla, CA). 8-BrcAMP, cortisol, corticosterone 22(*R*)-hydroxycholesterol, pregnenolone, 17β-oestradiol and testosterone were purchased from Sigma (St. Louis, MO). Dexamethasone was from UVA (Paris, France), RU 38486 was kindly provided by Roussel-Uclaf (Romainville, France).

Results

Under the culture conditions used in the present study, no significant effect of exogenous steroids on the number of cells was observed (data not shown). The amount of corticosteroids produced by cultured adrenocortical cells incubated in the presence of $ACTH_{1-24}$ for 30 min to 3 h is shown in Fig. 1. The pattern of secretion was sigmoidal, with the most rapid increase occurring between 60 and 90 min. After exposure of cells to 10^{-6} M dexamethasone for 24 h, the pattern of steroid output was quite similar, but at every time the amount of corticosteroids produced was higher than for control cells; the mean per cent increase over control cells was 173 ± 19 ($P < 0.05$).

The positive effect of dexamethasone on $ACTH_{1-24}$-induced steroid output of adrenal cells was not observed when the duration of the treatment was equal to or lower than 5 h (Fig. 2). The maximal effect was achieved after 30 h of culture in the presence of dexamethasone ($P < 0.01$). For longer treatments, the response to dexamethasone decreased steadily and even became not significant ($P > 0.05$) at 70 h.

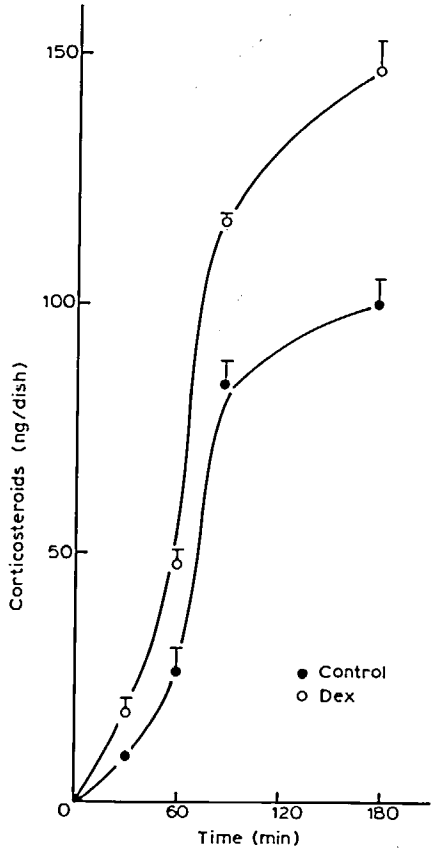

Fig. 1. Time-course of $ACTH_{1-24}$-induced corticosteroid production by control and dexamethasone-treated cultured ovine adrenocortical cells. Arenocortical cells were cultured for 25 h in the absence (●) or presence (○) of 10^{-7} M dexamethasone. On day 3, cells were incubated with 10^{-10} M $ACTH_{1-24}$ for various periods of time. Each point is the mean ± S.E.M. of triplicate determinations for three different wells.

The enhancing effect observed after optimal time of treatment showed a clear dependence upon both the molecular species and the concentration of the steroid used (Fig. 3). Cells exposed to 10^{-8} to 10^{-5} M dexamethasone released more corticosteroids in response to $ACTH_{1-24}$ than did control cells ($P < 0.05$ to $P < 0.001$) . However, the effect of dexamethasone concentrations was biphasic. The maximal secretion of corticosteroids was observed when cells were cultured in the presence of 10^{-7} M dexamethasone. With higher

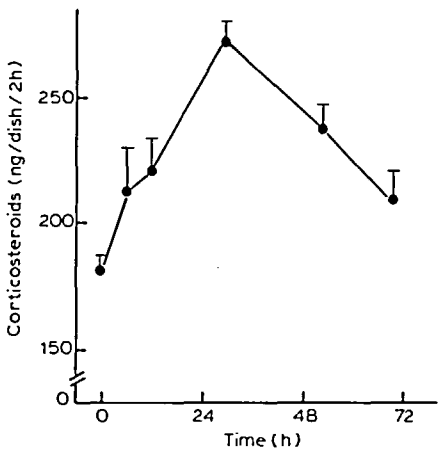

Fig. 2. Influence of the duration of treatment with dexamethasone on $ACTH_{1-24}$-induced steroidogenesis of adrenocortical cells. After various times of culture in the presence of 10^{-6} M dexamethasone, adrenocortical cells were incubated for 2 h in the presence of 10^{-10} M $ACTH_{1-24}$ and their production of corticosteroids was assayed as described in Materials and Methods. Each point in the mean ± S.E.M. of triplicate determinations for three different wells.

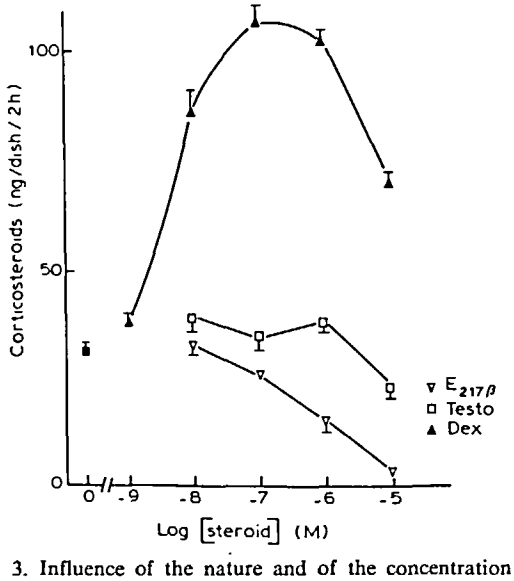

Fig. 3. Influence of the nature and of the concentration of several steroids on $ACTH_{1-24}$-induced steroidogenesis. Cells were cultured for 24 h in the absence (■) or presence of different concentrations of dexamethasone (▲), testosterone (□) or 17β-oestradiol (△). On day 3, cells were incubated for 2 h in the presence of $5 \cdot 10^{-10}$ M $ACTH_{1-24}$ and corticosteroid content of the medium was determined. Each point is the mean ± S.E.M. of triplicate determinations for three different wells.

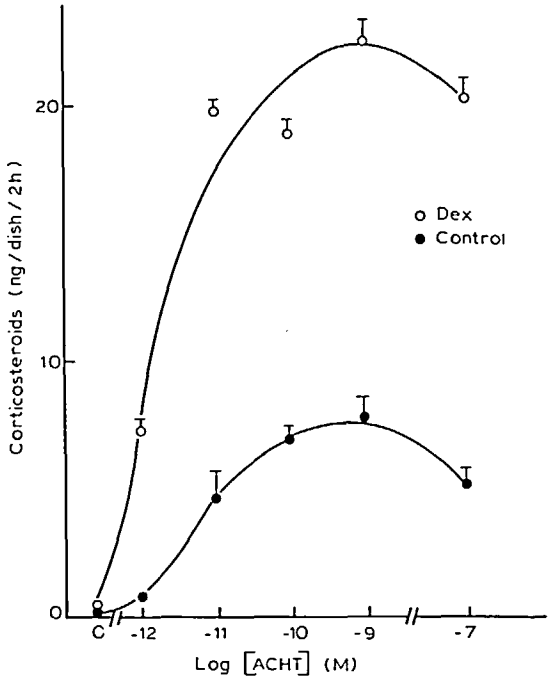

Fig. 4. Effect of increasing concentrations of $ACTH_{1-24}$ on the production of corticosteroids by adrenocortical cells cultured for 24 h in the absence (●) or presence (○) of 10^{-6} M dexamethasone. Each point is the mean ± S.E.M. of triplicate determination for three different wells.

concentrations the steroidogenic response to $ACTH_{1-24}$ decreased; cells exposed to 10^{-5} M dexamethasone produced only 64% ($P < 0.01$) of the amount of corticosteroids produced by cells cultured in the presence of 10^{-7} M dexamethasone. The ED_{50} of dexamethasone calculated on six independent experiments was 2.8 ± 0.8 10^{-9} M (mean ± S.E.M.). Testosterone and 17β-oestradiol were not able to increase the steroidogenic response to $ACTH_{1-24}$ of adrenocortical cells. Conversely, 17β-oestradiol at the two higher concentrations tested exhibited a negative effect on $ACTH_{1-24}$-induced steroidogenesis ($P < 0.02$ and $P < 0.01$) and this effect was clearly dose-dependent. The natural glucocorticoids cortisol and corticosterone also enhanced $ACTH_{1-24}$-induced steroidogenesis. The magnitude of their maximal effect was similar to that of dexamethasone, but it was achieved with concentrations roughly 100-fold higher than for dexamethasone (data not shown).

Exposure of adrenocortical cells to 10^{-6} M dexamethasone did not affect their basal secretion of corticosteroids (Fig. 4). However, such a treatment resulted in an enhancement of the steroid production in response to every concentration of $ACTH_{1-24}$ over that of control cells ($P < 0.01$ to $P < 0.001$). The mean per cent increase at $(1–5) \cdot 10^{-10}$ M $ACTH_{1-24}$ was 277 ± 39 (range 149–466) ($P < 0.01$) for nine separate experiments. Moreover, the ED_{50} of ACTH was slightly decreased from $9 \cdot 10^{-12}$ to $3 \cdot 10^{-12}$ M ($P < 0.01$) for six experiments.

RU 38486, a potent antiglucocorticoid [22] did not modify the glucocorticoid response to $ACTH_{1-24}$ when applied to adrenocortical cells for 24 h (between day 2 and 3) in concentrations between 10^{-8} and 10^{-6} M (Fig. 5A). However, $ACTH_{1-24}$-induced steroidogenesis of cells exposed to 10^{-5} M RU 38486 was 3-fold lower ($P < 0.01$) than that of control cells. In addition, increasing concentrations of RU 38486 antagonized the enhancing effect of 10^{-7} M dexamethasone in such a way that at 10^{-6} M RU 38486, the effect of dexamethasone was completely abolished. On day 6 of culture, the number of cells counted in culture dishes receiving 10^{-6} M RU 38486 for 48 to 120 h was significantly higher than in control wells. This effect of RU 38486 was clearly time-dependent (data not shown). Hence the results obtained in the following experiments were corrected for the number of cells.

Adrenocortical cells exposed to 10^{-6} M RU 38486 for 72 h exhibited, on day 6 of the experiment, a basal output of steroids significantly lower than that of control cells ($P < 0.05$) (Fig. 5B). $ACTH_{1-24}$-induced steroidogenesis also was decreased by RU 38486 and this effect was clearly dependent on the duration of the treatment (Fig. 5B). A 2 h exposure to RU 38486 did not modify the steroidogenic response to $ACTH_{1-24}$, but after 24 h the corticosteroid output was only 64% of that of control cells ($P < 0.02$). The response to $ACTH_{1-24}$ decreased again after 48 and 72 h of exposure to RU 38486 then remained low and

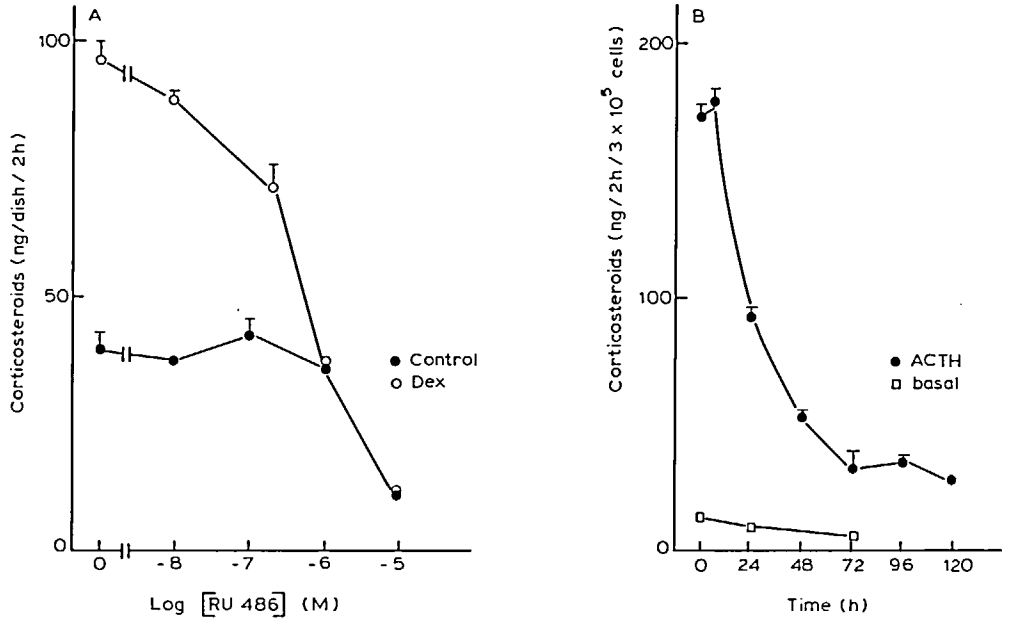

Fig. 5. Effect of RU 38486 on basal and $ACTH_{1-24}$-induced steroidogenesis of cultured ovine adrenocortical cells. (A) Cells were cultured for 24 h with different concentrations of RU 38486 (or RU 486), and in the absence (●) or in the presence (○) or 10^{-7} M dexamethasone. On day 3, cells were incubated for 2 h with $5 \cdot 10^{-10}$ M $ACTH_{1-24}$, and the amount of corticosteroids accumulated in the medium was determined. (B) Cells were cultured for various times in the presence of 10^{-6} M RU 38486 then incubated for 2 h in the absence (□) or presence (●) of $5 \cdot 10^{-10}$ M $ACTH_{1-24}$ and the amount of corticosteroids accumulated in the medium was determined. Each point is the mean ± S.E.M. of triplicate determinations for three different wells.

TABLE I

EFFECT OF DEXAMETHASONE ON BOTH cAMP AND CORTICOSTEROID OUTPUTS OF OVINE ADRENOCORTICAL CELLS STIMULATED BY $ACTH_{1-24}$ FORSKOLIN OR 8-BrcAMP

Adrenocortical cells were cultured for 24 h in the absence or presence of 10^{-6} M dexamethasone (Dex). On day 3, the medium was replaced by fresh medium containing 0,5 mM 1-methyl-3-isobutyxanthine and with either no addition or with addition of $ACTH_{1-24}$, forskolin or 8-BrcAMP, and corticosteroid productions during 2 h were assessed. Each value is the mean ± S.E.M. of triplicate determinations for three different dishes.

Stimulating factor	cAMP (pmoles per dish)		Corticosteroids (ng per dish)	
	−Dex	+Dex	−Dex	+Dex
Basal	0.6 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
$ACTH_{1-24}$ (10^{-12} M)	1.4 ± 0.1	1.5 ± 0.1	3.3 ± 0.1	12.7 ± 0.7 [a]
$ACTH_{1-24}$ (10^{-10} M)	89 ± 3	119 ± 10 [a]	117 ± 2	204 ± 2 [a]
$ACTH_{1-24}$ (10^{-8} M)	151 ± 12	179 ± 12	130 ± 5	191 ± 12 [a]
Forskolin (10^{-5} M)	33 ± 2	30 ± 1	115 ± 10	195 ± 12 [a]
8-BrcAMP (10^{-3} M)	n.d. [b]	n.d. [b]	51 ± 1	84 ± 4 [a]

[a] $P < 0.05$ to 0.001 vs. corresponding controls (cells not exposed to dexamethasone).
[b] n.d., not determined.

constant until 120 h where the response of treated cells was only 32% of that of control cells ($P < 0.001$).

In order to learn more about the mechanism of glucocorticoid enhancement of adrenal steroidogenesis, we investigated next whether dexamethasone enhanced the response of adrenocortical cells only to $ACTH_{1-24}$ or also to other stimulating factors. It is shown in Table I that a treatment with dexamethasone enhanced $ACTH_{1-24}$-induced both cAMP and corticosteroid outputs, forskolin induced steroidogenesis, but not forskolin-induced cAMP output and also 8-BrcAMP-induced steroidogenesis. It should also be noted that as for $ACTH_{1-24}$ the enhancement by dexamethasone of forskolin or 8-BrcAMP-induced steroidogenesis was antagonized by RU 38486 and that the ED_{50} of dexamethasone was similar for $ACTH_{1-24}$- or 8-BrcAMP-induced steroidogenesis. Also, the output of corticosteroids in response to 8-BrcAMP of cells exposed to 10^{-5} M dexamethasone was significantly lower than that of cells exposed to 10^{-7} M dexamethasone (data no shown).

In an attempt to roughly localize the enzymatic step(s) located beyond cAMP involved in this effect of glucocorticoids, we studied the production of corticosteroids by control and dexamethasone-treated adrenocortical cells incubated either in the presence of 22(*R*)-hydroxycholesterol (Table II) or of exogenous pregnenolone. In both cases, the amounts of corticosteroids released by control and dexamethasone-treated cells were similar.

Next, we studied whether the presence of insulin or of horse serum in the culture/incubation medium was necessary to express this effect. Fig. 6 shows that in the presence of 2% horse serum,

TABLE II

EFFECT OF DEXAMETHASONE ON CORTICOSTEROID PRODUCTION OF ADRENOCORTICAL CELLS STIMULATED BY $ACTH_{1-24}$ OR INCUBATED IN THE PRESENCE OF 22(*R*)-HYDROXYCHOLESTEROL OR EXOGENOUS PREGNENOLONE

Adrenocortical cells were cultured for 25 h in the absence or presence of 10^{-6} M dexamethasone (Dex). On day 3, corticosteroid production during a 2 h period was assessed under several conditions. Each value is the mean ± S.E.M. of triplicate determinations for three different wells.

Incubation	Corticosteroids (ng per dish)	
	−Dex	+Dex
Basal	11.4 ± 1.1	14.2 ± 1.0
$ACTH_{1-24}$ (10^{-10} M)	117 ± 2	204 ± 1 [a]
22(*R*)-hydroxycholesterol $4 \cdot 10^{-6}$ M	152 ± 5	137 ± 7
22(*R*)-hydroxycholesterol $12 \cdot 10^{-6}$ M	208 ± 6	174 ± 3
Pregnenolone $6.3 \cdot 10^{-7}$ M	100 ± 5	113 ± 1
Pregnenolone $1.9 \cdot 10^{-6}$ M	233 ± 9	223 ± 17

[a] $P < 0.001$ vs. cells cultured in the absence of dexamethasone.

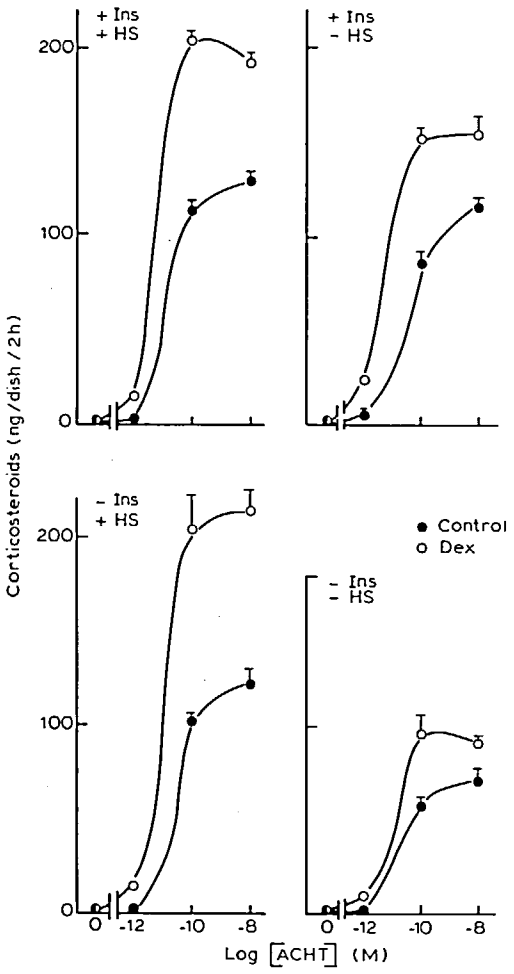

Fig. 6. Influence of various conditions of culture on the enhancing effect of dexamethasone on $ACTH_{1-24}$-induced steroidogenesis of adrenocortical cells. Cells were cultured in the absence of horse serum (−HS) with (+Ins) or without (−Ins) insulin or in presence of horse serum (+HS) with or without insulin for 72 h, and exposed for 24 h to 10^{-6} M dexamethasone (o) or maintained in the absence of the steroi (•). On day 3, cells were incubated for 2 h in the presence of increasing concentrations of $ACTH_{1-24}$, and the amount of corticosteroids accumulated in the medium was determined. Each point is the mean ± S.E.M. of triplicate determination for three different wells.

10^{-6} M dexamethasone, enhanced $ACTH_{1-24}$-induced steroidogenesis to a similar extent in the presence or absence of insulin. When the culture was performed in the presence of insulin, but in the absence of horse serum, the steroidogenic response to $ACTH_{1-24}$ of both control and dexamethasone-treated cells was lower than that of corresponding cells cultured with serum ($P < 0.05$ to $P < 0.01$). However, the magnitude of the relative enhancement by dexamethasone of the steroidogenic response to $ACTH_{1-24}$ was identical in the absence or presence of serum. Omission of insulin in serum-free culture medium resulted in a decreased steroid output at every concentration of $ACTH_{1-24}$ tested ($P < 0.05$ to $P < 0.01$ vs. cells cultured without serum with insulin). However, the relative increase by dexamethasone was quite similar to that observed in the presence of insulin. It is also worthy to note that dexamethasone enhanced the cAMP response to $ACTH_{1-24}$ to the same extent under these four conditions of culture (data not shown).

Discussion

The present study indicates that glucocorticoids can modulate positively ovine adrenocortical steroidogenesis by a direct action on fasciculata cells. However, the pathway which mediates this effect is not yet clear. Heterogeneity of steroid hormone-binding sites in target tissues is a well-recognized phenomenon [23–28]. The glucocorticosteroid effect described here exhibited the following characteristics: (i) it required more than 5 h to be expressed; (ii) it was specific for steroids having a glucocorticoid activity and was antagonized by RU 38486 a potent antiglucocorticoid [22]; (iii) it was dose-dependent, the ED_{50} of dexamethasone being close to 3 nM; (iv) it was observed both in the absence and presence of serum in the culture/incubation medium; and (v) it affected not only $ACTH_{1-24}$ but also 8-BrcAMP-induced steroidogenesis. Taken together these results suggest that the effect of glucocorticosteroids we observed is not due to a 'non-specific membrane effect' but more likely involves binding of glucocorticosteroid molecules to some specific high-affinity receptors present in adrenal cells. If so, this might explain the lower steroidogenic response of cells cultured for 48 or 72 h to dexamethasone as compared to that of cells exposed to the steroid for only 30 h. Indeed, a lot of reports now document that glucocorticoids down-regulate their own receptors in many systems and that marked similaries are observed between the different models studied (for a review see Ref. 29).

Under our experimental conditions, cortisol and corticosterone were roughly 100-fold less potent than dexamethasone. This correlates closely with previous results on the enhancement of ACTH-induced cAMP production of adrenocortical cells [19]. Nevertheless, since high concentrations ($\geqslant 10^{-5}$ M) of steroids may be expected to accumulate in steroidogenic cells both in culture and in vivo [17,30,31] it is likely that the phenomenon described here is physiologically relevant. Such an assumption is strengthened by the lower steroid output, both under basal condition and when stimulated by $ACTH_{1-24}$ of adrenal cells cultured for 72 h in the presence of 10^{-6} M RU 38486, as compared to control cells. Indeed, this result most probably indicates that cortisol and corticosterone, which are the main steroids produced by ovine adrenocortical cells in culture [32,33], are involved in a paracrine and/or autocrine process which is necessary to the full steroidogenic activity of these cells. Talking of this, it seems worthy of noting that RU 38486 has been shown to produce a dose-dependent decrease in the activity of several enzymes of the corticosteroidogenic pathway, when given for 7 days to castrated, hypophysectomized/ACTH-replaced rats [34]. In addition, in the ovine fetus, ACTH-induced activation of adrenal function in vivo is attenuated by concurrent metyrapone infusion [35], whereas dexamethasone treatment for 48 h enhances the adrenal steroidogenic response to ACTH [36].

The mechanism of glucocorticoid action on steroidogenesis remains unknown. Exposure of cultured adrenocortical cells to glucocorticoids enhanced the response to $ACTH_{1-24}$ of their adenylate cyclase system (Ref. 19 and present results). On the other hand, it is clear from the above studies, that additional steps, located beyond cAMP but before pregnenolone formation, respond to dexamethasone treatment.

However, the effect of glucocorticoids could be expressed through direct actions on the adenylate cyclase system and on post-cAMP steps or, alternatively, it could be mediated through an enhancement of the responsiveness of adrenal cells to other hormone(s) or factor(s) (present in the culture/incubation medium) controlling these steps. Insulin at the concentration we used (10 μg/ml) binds not only to adrenal insulin receptors but also to IGFI receptors [37] and glucocorticoids have been shown to enhance both insulin and IGFI receptors in several tissues [38–41]. Now, both insulin and IGFI increase $ACTH_{1-24}$-, but not forskolin-induced cAMP output of adrenocortical cells and enhance their steroidogenic response to $ACTH_{1-24}$ or forskolin but not to 22(R)-hydroxycholesterol (Ref. 37 and unpublished results). Further, it has been reported that insulin in the adrenal gland [42] and both insulin and IGFI in granulosa cells [43,44] modulate low-density lipoprotein metabolism, and that one of the effects of serum present in culture media is to provide adrenal cells with cholesterol-rich lipoproteins [21,45]. Hence, we decided to study whether removing insulin and/or serum from the culture medium could modify the effect of dexamethasone. It was observed that even in the absence of insulin and serum, the relative intensity of the enhancing effect of dexamethasone on both $ACTH_{1-24}$-induced cAMP output and steroidogenesis was preserved.

Many groups have reported an inhibitory effect of rather high concentrations of glucocorticoids on ACTH-induced adrenal steroidogenesis [1–11,16]. This appears to result from both acute [3,4,6,8,9,10] and chronic [5,7,11,16] actions of the steroids. In the present study, only when concentrations of dexamethasone were raised above 10^{-6} M, a phenomenon which resembles this inhibitory effect could be observed. Taken together, these results reinforce the view that glucocorticoids are involved in a short-loop feed-back on adrenocortical cells, and that according to their concentration they can regulate in a biphasic manner adrenal steroidogenesis.

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