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STEROIDOGENESIS IN GUINEA PIG ADRENAL CORTEX: EFFECTS OF ACTH ON STEROID SECRETION AND STEROIDOGENIC ENZYME ACTIVITIES AND EXPRESSION

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Summary—In this study, we investigated guinea pig adrenal steroidogenesis, specially, C19 steroid production. Analysis of adrenal steroids by high performance liquid chromatography and gas chromatography indicated the presence of androstenedione and 11β-hydroxyandrostenedione. Adrenal androstenedione and 11β-hydroxyandrostenedione levels were stimulated by ACTH administration while only 11β-hydroxyandrostenedione was increased in plasma. *In vitro* studies using adrenal cortex cells in primary culture confirmed that 11β-hydroxyandrostenedione is the major C19 steroid secreted. The chronic treatment of guinea pig with ACTH stimulated all adrenal post-pregnenolone enzyme activities and decreased *P*450c21 and *P*450c17 mRNA levels while *P*450scc, 3β-hydroxysteroid dehydrogenase and *P*450c11 mRNAs remained unaffected. Treatment of adrenal cells in primary culture with ACTH for 72 h changed the distribution of steroids secreted and decreased 21-hydroxylase activity while 17α-hydroxylase and 17,20-lyase activities were increased favoring C19 steroid production. In ACTH-treated cells, the mRNA levels for *P*450c21 and *P*450c17 increased and reached a peak at 18 h. Our data indicate that treatment with ACTH stimulates adrenal steroidogenic capacity by increasing steroid secretion and causes transcriptional and post-transcriptional effects on steroidogenic enzymes gene expression. Finally, the direct action of steroids on steroid production by adrenal cells in primary culture was investigated. Our data indicate that steroids themselves increase C19 steroid synthesis and inhibit glucocorticoid production without affecting gene expression for steroidogenic enzymes.

INTRODUCTION

The human adrenal gland secretes C19 steroids, namely, dehydroepiandrosterone, its sulfate, 5-androstene-3β,17β-diol and androstenedione. These steroids circulate at concentrations higher than those of all other steroid hormones and recent evidence indicates that they exert androgenic activity in several tissues by conversion into potent androgens [1–6]. Despite this important role of adrenal C19 steroids, it is not yet clear how the secretion of these steroids is regulated. In this report, we present work from our laboratory undertaken in an attempt to better understand the control of C19 steroids by the adrenal.

The model chosen for this study was the adrenal cortex of the guinea pig. Previous studies have indicated that the guinea pig adrenal possesses the steroidogenic enzymes needed for the production of C19 steroids [7–10]: cholesterol side-chain cleavage (*P*450scc), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α-hydroxylase (*P*450c17), 21-hydroxylase (*P*450c21) and 11β-hydroxylase (*P*450c11). The following review summarizes our data on the concentration of steroids in guinea pig adrenal cortex, the capability of adrenal cells to produce and to secrete steroids, the steroidogenic enzyme activities and the steady state levels of mRNAs for 3β-HSD *P*450scc, *P*450c17, *P*450c11 and *P*450c21 under basal conditions and after treatment with ACTH both *in vivo* and *in vitro* using zona fasciculata-glomerulosa cells in primary culture. Finally, the direct action of steroids themselves on steroid secretion and steroidogenic enzyme activities of adrenal cells was also examined.

MATERIALS AND METHODS

Animals

Adult male guinea pigs (Hartley) weighing 650–700 g were obtained from Charles River

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Canada, Inc. (St. Constant, Quebec, Canada). Animals were kept at a controlled temperature of 22–25°C with a light period from 0500 to 1900 h and given food and water *ad libitum*. Blood from animals was collected and plasma samples, obtained after centrifugation at 1000 *g* were frozen at −20°C until steroid measurement. Adrenal glands were quickly removed and trimmed of fat using sterile instruments. Adrenals were cooled on ice and weighed before freezing at −20°C until steroid measurement. For mRNA analysis, adrenals were frozen on nitrogen and stored at −80°C.

Chemicals

Fetal calf serum was obtained from Hyclone Labs (Logan, UT, U.S.A.). Twenty-four-well culture plates and 100 × 20 mm Petri dishes were obtained from Flow Labs (McLean, VA, U.S.A.). The synthetic tetracosapeptide ACTH (Cortrosyn) was purchased from Organon (West Orange, NJ, U.S.A.). [1,2,6,7-^{3}H]-Progesterone (81 Ci/mmol) and [1,2,6,7-^{3}H]-17α-hydroxyprogesterone (74 Ci/mmol) were purchased from Amersham (Oakville, Ontario, Canada). [7-^{3}H]Pregnenolone (24 Ci/mmol) and [1,2-^{3}H]deoxycortisol (42 Ci/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). Pregnenolone, progesterone, 17α-hydroxyprogesterone and deoxycortisol were purchased from Steraloids Inc. (Wilton, NH, U.S.A.).

Isolation of adrenal cells and steroidogenic enzyme activity

The adrenal cells were isolated using an adaptation [11] of the procedure described by Black *et al.* [9]. After isolation, adrenal cells were plated in 24-well Petri dishes in 1 ml Eagle's Minimum Essential Medium (MEM) supplemented with 12% dextran-coated charcoal-treated fetal calf serum (v/v), 100,000 U/l penicillin, 50 mg/l streptomycin and $NaHCO_3$ (2.2 g/l) (MEMS) at a density of 2.5×10^5 adrenal cells/ml in order to determine enzymatic activity. Cells were plated at a density of 1.25×10^6 cells/ml in 10 ml MEMS in 100 × 20 mm Petri dishes for the measurements of mRNA levels and steroid secretion. Cells were incubated under 5% carbon dioxide in humidified air at 37°C. Twenty-four hours after plating, the medium was changed and the experiments were started.

Enzymatic activity

At the time of harvest, the medium was removed and cells were incubated for 15 min in MEMS alone. The enzyme activities were then assayed as described previously [12]. Briefly, 1 ml of MEMS containing tritiated pregnenolone (0.36 Ci) and pregnenolone (1 μM) was incubated for 30 min for the 3β-HSD assay. Values for 3β-HSD activity were obtained by calculating the conversion of pregnenolone to progesterone, 17α-hydroxyprogesterone, deoxycorticosterone, corticosterone, deoxycortisol, cortisol, androstenedione and 11β-hydroxyandrostenedione. One milliliter of MEMS containing tritiated progesterone (0.36 Ci) and progesterone (1 μM) was incubated for 60 min for the 17α-hydroxylase assay. Values for 17α-hydroxylase activity were obtained by calculating the conversion of progesterone to 17α-hydroxyprogesterone, deoxycortisol, cortisol, androstenedione and 11β-hydroxyandrostenedione. One milliliter of MEMS containing tritiated 17α-hydroxyprogesterone (0.36 Ci) and 17α-hydroxyprogesterone (1 μM) was incubated for 30 min for the 21-hydroxylase and 17,20-lyase assays. Values for 21-hydroxylase and 17,20-lyase activities were obtained by calculating the conversion of 17α-hydroxyprogesterone to deoxycortisol and cortisol for 21-hydroxylase activity and androstenedione and 11β-hydroxyandrostenedione for 17,20-lyase activity. One milliliter of MEMS containing tritiated deoxycortisol (0.36 Ci) and deoxycortisol (1 μM) was incubated with adrenal cells for 60 min for the 11β-hydroxylase assay. Values for 11β-hydroxylase activity were obtained by calculating the conversion of deoxycortisol to cortisol.

The enzymatic reaction was stopped by adding 50 μl of 1N acetic acid to cells. The medium was then recovered and frozen at −20°C until assayed. The products of metabolism were assayed as follows. The medium was extracted twice with 5 ml ethyl ether–acetone (1:1, v/v). The extract was evaporated to dryness and the remaining residue dissolved in a mixture of methanol–water (1:1, v/v) subjected to high performance liquid chromatography (HPLC) analysis using a Waters model 510 chromatograph (Waters Instruments, Milford, MA, U.S.A.) and a C18 column (Radial-Pak, Waters). A gradient of 100% methanol–water

(1:1, v/v) to 41% tetrahydrofuran–acetonitrile (1:1, v/v), over a 35 min period, was used for the analysis of pregnenolone, progesterone, 17α-hydroxyprogesterone and deoxycortisol metabolism. The formation of tritiated metabolites was quantitated by integrating and radioactive peaks and divided by the sum of total radioactivity recovered after chromatography. Results were then expressed as percent conversion while enzyme activity was expressed in pmol/10^6 cells/h for all steroids assayed. Enzyme activities were assayed in triplicate. For each steroid enzyme activity measured, the rate of conversion of steroid substrate was linear for the times indicated.

Steroid determination

At the time of harvest, the medium was centrifuged at 200 *g* for 5 min at 4°C and the supernatant frozen until assayed. Plasma and medium levels of steroids were determined in 1 ml samples by radioimmunoassays (RIA) after ether extraction and chromatography on Amprep C-18 columns (Amersham, Oakville, Ontario, Canada) and LH-20 columns (Pharmacia, Uppsala, Sweden) as described previously [12, 13]. The frozen adrenals were homogenized and extracted in organic solvent. Steroids were separated by chromatography on C-18 and LH-20 columns and non-conjugated and sulfoconjugated steroids levels were determined by RIA as described previously [12, 13].

mRNA preparation and analysis (Northern blot analysis)

Three adrenals from three different guinea pigs were homogenized with a Polytron (Brinkmann Instruments, Westbury, NY, U.S.A.) in 2 ml of 4 M guanidinium thiocyanate, 100 mM 2-mercaptoethanol, 2% *N*-lauryl sarcosine, 50 mM Tris–HCl (pH 7.5) and 50 mM EDTA. The homogenates were centrifuged at 1000 *g* at 10°C for 30 min. The supernatant was centrifuged through 5.7 M CsCl [14]. The RNA pellets obtained were dissolved in 0.5% sodium dodecyl sulfate (SDS) and 10 mM Tris–HCl (pH 7.5), and then precipitated twice with ethanol. The total RNA obtained was quantitated by spectrophotometry at 260 nm; 300 to 400 μg of total RNA were recovered from one adrenal gland. The ratio of absorbance at 260 vs 280 nm was always greater than 1.85.

For Northern blots, 50 μg total cellular mRNA per lane was denatured by heating 1 h at 50°C using glyoxal. RNA was then electrophoresed, transferred to nylon membranes (Hybond-N, Amersham, Arlington Heights, IL, U.S.A.) in 20 × SCC (1 × SCC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) overnight at 25°C. RNA was immobilized covalently by cross-linking under short wavelength u.v. for 3 min [15, 16]. Prehybridization was performed for 12 h at 42°C in 50% formamide, 6 × SSC, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin (BSA), 1% SDS, 200 μg/ml heat denatured salmon sperm DNA and 200 μg/ml yeast tRNA. Hybridization was then performed by adding the probe to the prehybridization solution and incubation was continued for 24 h. Filters were then washed in 0.1 × SSC with 1% SDS for 20 min at room temperature, followed by three 20-min washes in the same solution at 65°C.

For RNA Dot blot hydridization assays, RNA was denaturated by heating at 65°C for 15 min in 6 × SCC containing 7.5% formaldehyde. Serial 2-fold dilutions of RNA in 10 × SCC were spotted onto nylon membranes using a 96-well Hybri-dot Manifold (Bethesda Research Labs, Gaithersburg, MD, U.S.A.). Dot blots were processed as described above for Northern blots. The Northern and Dot blots were then autoradiographed at −80°C with intensifying screens.

The intensities of the autoradiographic spots were quantified using an Amersham RAS Image Analyzer system. The slopes of the dot intensities of each dilution series were calculated by linear regression using Cricket Graph (Cricket Software, Malvern, PA, U.S.A.).

Probes

For Northern blots, the same blot was successively probed by 5 cDNAs and, for Dot blot, the same Dot blot was only probed by 3 cDNAs. Full-length human *P*450scc [17] and human *P*450c17 [18] cDNAs were isolated from plasmid pUC18 by cleavage with *Eco*RI. The *P*450c21 probe was a 1200 base pair fragment from human *P*450c21 cDNA [19] isolated after subcloning into the *Eco*RI site of plasmid pBR 322. The *P*450scc, *P*450c21 and *P*450c17 probes were generously provided by Dr Walter L. Miller. The 3β-HSD probe (generously provided by Dr Fernand Labrie) was a 1038 base pair *Eco*RI/PVU II fragment of human

3β-HSD cDNA [20] which was isolated from plasmid pT7T3/18U. The *P*450c11 probe (generously provided by Dr Perrin C. White) was a 2000 base pair *Eco*RI/*Bam* H1 fragment of human pH11F3b cDNA [21] isolated after subcloning from plasmid Bluescript KS^+. Each digested fragment was separated on agarose gel electrophoresis and the cDNA inserts were purified on Na45 membrane. Probes were labeled with [^{32}P]dCTP to 10^9 dpm/μg using random primer kits purchased from Amersham. After the fifth hybridization on Northern blot, the first hybridization was repeated and no more than 15% of the signal was lost.

Data analyses

RIA data were analyzed using a program based on model II of Rodbard and Lewald [22]. Experimental data are presented as mean ± SEM and statistical significance was measured according to the Duncan–Kramer multiple-range test [23].

RESULTS

Effects of ACTH on adrenal and plasma steroid levels

The retention times on HPLC and the mass spectra obtained by selected ions were consistent with the presence in the guinea pig adrenals of pregnenolone, progesterone, 17α-hydroxyprogesterone, androstenedione, 11β-hydroxyandrostenedione and cortisol [24]. Two hours after a single injection of ACTH the levels of steroids in the guinea pig adrenals increased (Table 1). However, in plasma, only pregnenolone, 11β-hydroxyandrostenedione, androstane-3α,17β-diol, corticosterone and cortisol concentrations were affected.

Effects of in vivo chronic ACTH treatment on adrenal and plasma steroids and the activity of adrenal steroidogenic enzymes and their mRNA levels

Chronic administration of ACTH for 7 days produced no significant difference in the adrenal content of steroids between the ACTH-treated group sacrificed 8 h after the last injection and the control group [25]. However, a marked decrease in the adrenal content of C21 and C19 steroids was observed in the group sacrificed 24 h after the last injection of ACTH. Of the plasma C19 steroids examined, only 11β-hydroxyandrostenedione levels were still increased 8 h after the last injection of ACTH while plasma levels of C21 steroids were increased. At 24 h after the last injection of ACTH, plasma steroid levels were returned to basal values. 3β-HSD, 17α-hydroxylase, 17,20-lyase, 21-hydroxylase and 11β-hydroxylase activities increased significantly in a suspension of adrenal cells obtained from ACTH-treated animals [25]. ACTH-treatment also caused a marked decrease of *P*450c21 and *P*450c17 mRNA levels while *P*450scc, 3β-HSD and *P*450c11 mRNA levels remained unaffected [25].

Effects of ACTH on guinea pig adrenal cells in primary culture

Treatment with ACTH for 24 h of adrenal cells in primary culture demonstrated an ACTH concentration-dependent stimulation of all steroids measured [11]. A steroidogenic response was observed with the half-maximal effect occurring at ACTH concentrations varying between 1.7×10^{-11} and 1.1×10^{-10} M for the 12 steroids measured with no significant difference. Incubation of adrenal cells

Table 1. Basal and stimulated (ACTH) adrenal and plasma steroid levels in castrated guinea pigs

	Control	ACTH-treated	Control	ACTH-treated
Steroids	(fmol/mg adrenal)		(pmol/ml plasma)	
Pregnenolone	3123 ± 934	8245 ± 767	2.14 ± 0.71	4.90 ± 0.74
Progesterone	537 ± 79	1812 ± 265	ND	ND
17α-Hydroxypregnenolone	144 ± 15	467 ± 21	ND	ND
17α-Hydroxyprogesterone	557 ± 188	6505 ± 1108	ND	ND
Dehydroepiandrosterone	35 ± 3	53 ± 7	ND	ND
5-Androstene-3β,17β-diol	15 ± 1	22 ± 3	ND	ND
Androstenedione	87 ± 11	534 ± 45	ND	ND
11β-Hydroxyandrostenedione	381 ± 26	3234 ± 398	32.6 ± 7.5	79.1 ± 1.5
Testosterone	8 ± 1	82 ± 9	ND	ND
Androstane-3α,17β-diol	20 ± 1	33 ± 1	ND	ND
Corticosterone	395 ± 55	1311 ± 103	13.2 ± 1.7	36.9 ± 4.0
Cortisol	3089 ± 301	53,502 ± 4423	297 ± 104	510 ± 40

Experiments were performed two times; the results are those of one representative experiment. ND: under the limit of detection. This table was taken from Ref. [24].

with 8Br-cAMP also increased the secretion of corticosterone, cortisol, androstenedione and 11β-hydroxyandrostenedione in a dose-dependent manner [11]. Since 11β-hydroxyandrostenedione appears to be the major C19 steroid secreted by the guinea pig adrenal cells, we carried out an experiment to determine its precursor. Adrenal cells were incubated for 48 h with either labeled corticosterone, cortisol, 11-deoxycorticosterone or androstenedione. Analysis by HPLC of the medium indicated that only androstenedione can be converted into 11β-hydroxyandrostenedione [11].

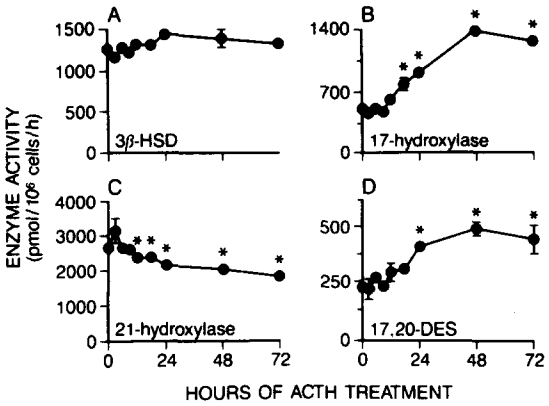

Fig. 1. Time course of the effect of 10 nM ACTH on steroidogenic enzyme activities in FG cells in primary culture. Results are expressed in pmol of metabolites formed per 10^6 cells per h. Data represent the mean ± SEM of duplicate wells. This figure was taken from Ref. [26].

Effect of ACTH on steroid secretion, activity of adrenal steroidogenic enzyme and their mRNA levels in adrenal cells in primary culture

The rate of steroid secretion by adrenal cells in the presence of 10 nM ACTH for a 72-h period remained constant throughout the first 24 h of incubation and they decreased after 48 h to approx. 60% of the maximal rate and remained at this level up to the last time interval studied [26]. In the first 12 h following the addition of ACTH, the production of 17-deoxy C21 steroids rapidly decreased and it remained at a plateau between 12 and 24 h, it further declined after 72 h of exposure to ACTH. Secretion of 17α-hydroxy C21 steroids which remained unchanged in the first 24 h of incubation with ACTH, diminished by 55% between 24 and 48 h. On the other hand, the production of 4-ene C19 steroids steadily increased between 3 and 48 h and remained elevated thereafter. Twelve to 48 h following exposure to ACTH, both 17α-hydroxylase and 17,20-lyase activities showed an increase while the activity of 21-hydroxylase steadily declined during incubation with ACTH (Fig. 1). The activity of 11β-hydroxylase in adrenal cells was not affected by ACTH treatment.

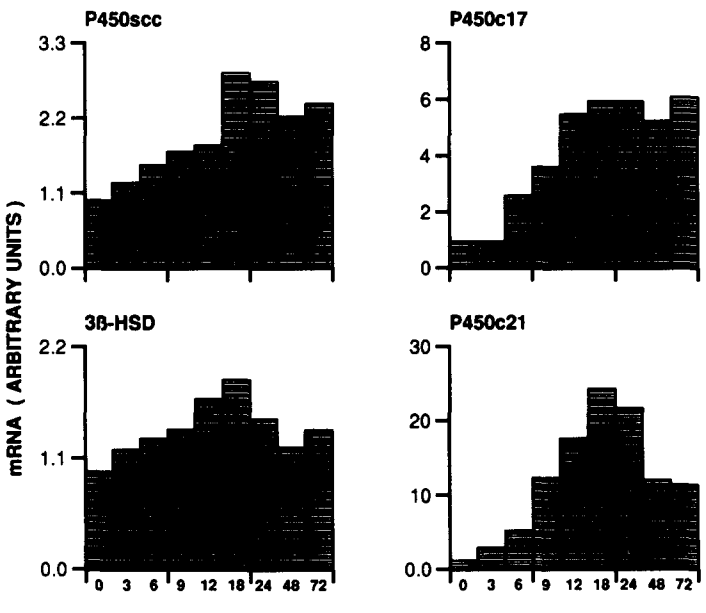

Fig. 2. Time course of the effect of 10 nM ATCH on mRNAs for *P*450scc, 3β-HSD, *P*450c21 and *P*450c17 levels in FG cells in primary culture. Each data point represents the relative amount of mRNA estimated by densitometry analysis of Northern blot analysis for each enzyme. Experiments were performed four times; the results are those of one representative experiment. This figure was taken from Ref. [26].

Northern blot analysis revealed that *P*450scc and 3β-HSD mRNA increased by 2- and 3-fold, respectively, after 18 h of ACTH treatment and declined thereafter to 2.4- and 1.4-fold, respectively, over control values after 72 h of treatment with ACTH (Fig. 2). ACTH induced a time-dependent increase in *P*450c21 mRNA, attaining a maximum increase of 24.5-fold after 18 h. *P*450c21 mRNA levels decreased thereafter, but still remained at levels higher than those of the control group. *P*450c17 mRNA reached a maximum of 6-fold increase after 18 h of ACTH treatment and remained at this level for the 72-h incubation period.

Effects of androstenedione and RU 486 [17β-hydroxy-11β-(4-dimethylamino phenyl) 17α-(1-propynyl)estra-4,9-dien-3-one] on steroidogenic enzymes in guinea pig adrenal cells

Previous studies have already showed that RU 486 may alter the activity of adrenal steroidogenic enzymes [27] and, to better investigate this inhibitory effect on steroidogenesis, we compared the action of RU 486 with that of androstenedione on adrenal steroidogenic enzyme activities [12, 28]. Androstenedione and RU 486 decreased 21-hydroxylase activity while only RU 486 increased the activities of 17α-hydroxylase and 17,20-lyase. It must be noted that the combined addition of androstenedione and ACTH further enhanced the decrease in 21-hydroxylase activity observed with androstenedione alone and, furthermore, caused a marked stimulation in 17α-hydroxylase and 17,20-lyase activities [28]. Treatment of adrenal cell cultures with androstenedione decreased 11β-hydroxylase activity to approx. 80% of the control value while RU 486 had no effect on this activity [12].

DISCUSSION

The present study showed that the major C19 steroid secreted by the guinea pig adrenal is 11β-hydroxyandrostenedione. In agreement with the *in vivo* data, we demonstrated that both C19 steroids and C21 steroids are produced by guinea pig adrenal cells in primary culture. In fact, dehydroepiandrosterone, androstenedione and 11β-hydroxyandrostenedione were generated, while dehydroepiandrosterone and androstenedione were rapidly converted into 11β-hydroxyandrostenedione. In adult human, the main adrenal C19 steroids are dehydroepiandrosterone and its sulfate, and most of the studies on adrenal steroidogenesis in rat, guinea pig, and dog were first focused on the secretion of these steroids [29]. The adrenal and plasma concentrations of dehydroepiandrosterone and its sulfate in these species are extremely low due to high activity of adrenal 3β-HSD which converts dehydroepiandrosterone into androstenedione [24, 29]. Our data demonstrate that, in the guinea pig adrenal, the steroidogenic pathway is shifted towards 4-ene-steroids and hydroxylated at position 11 of the C19 steroid formed.

The present study also showed a change in the distribution of steroids secreted by adrenal cells in culture during long-term stimulatory influence of ACTH. The production of cortisol, androstenedione and 11β-hydroxyandrostenedione steadily increased during the incubation period with ACTH due to a marked stimulation of both 17α-hydroxylase and 17,20-lyase activities. The decrease in 21-hydroxylase activity enhanced the shift of the steroidogenic secretion towards C19 steroids. mRNA levels of 3β-HSD, *P*450scc, *P*450c21 and *P*450c17 increased and reached a peak at 18 h and, with the exception of *P*450c17, dropped between 24–72 h after addition of ACTH. Our results revealed major differences in the time courses and levels of the induction of mRNAs and the activity of the steroidogenic enzymes in adrenal cells in culture. The stimulation of *P*450c17 showed similar time courses at both enzymatic activity and mRNA levels, while there was no relationship between enzymatic activity and mRNA levels for 3β-HSD, *P*450c21 and *P*450scc. The most striking difference was observed in the case of *P*450c21 whose enzymatic activity diminished by 20% while its mRNA level was stimulated 25-fold after 18 h of incubation and remained elevated throughout the period of exposure to ACTH. In bovine adrenal cells, differences in the induction by ACTH of *P*450c21 mRNA, protein synthesis and enzymatic activity were previously noted during long-term stimulation and it was concluded that other factor(s) must be present within the cells for the regulation of the enzyme activity [30]. Our observation that 3β-HSD mRNA levels also increased in the absence of any effect on its enzyme activity, suggests that similar mechanisms may also be involved in the activation of other steroidogenic enzymes.

Using the *in vitro* model of adrenal cells in primary culture, the present study also demonstrated an inhibitory effect of C19 steroids on

adrenal steroidogenic enzymes. Such an effect of C19 steroids was shown at basal as well as ACTH-stimulated levels. A short-loop negative feedback on steroid-transforming enzymes by steroids themselves is supported by our observation that androstenedione had no effect on basal and ACTH-stimulated steady-state *P*450c11 and *P*450c21 mRNA levels. In agreement with previous observations [31–33], our data reinforce the concept of the action of steroid themselves on steroidogenesis.

We also demonstrated that the administration of ACTH to intact guinea pigs induces an increase of all post-pregnenolone steroidogenic enzymes including 3β-HSD, 17α-hydroxylase, 17,20-lyase, 21-hydroxylase and 11β-hydroxylase. However, the increased steroid secretion and steroid enzyme activities observed in ACTH-treated guinea pigs were not associated with a rise in the levels of mRNA encoding for the enzymes. Chronic ACTH administration caused a significant decrease in *P*450c21 and *P*450c17 while *P*450450scc, 3β-HSD and *P*450c11 mRNA levels remained unaltered. Thus, in contrast to the *in vitro* situation, *in vivo*, a marked stimulatory effect of ACTH is observed on steroid-transforming enzyme activities whereas the mRNA levels may not be increased. The effects of ACTH in intact guinea pigs may be different from that observed in hypophysectomized animals where the adrenal gland undergoes atrophy. Our data may be interpreted as indicating that *in vivo* chronic treatment of guinea pigs with ACTH stimulates adrenal steroidogenic capacity by increasing steroid secretion and steroid enzyme activity. Furthermore, a post-transcriptional effect on steroidogenic enzymes gene expression by affecting the half-life of their mRNAs, protein stabilization or decrease protein turnover is also suggested [34]. In agreement with this observation, it was previously reported that the hypertrophy of the adrenal gland observed in ACTH-treated animals could be correlated with a change in protein and RNA half-life. [35]

In summary, the present data show that guinea pig adrenal produce and secrete C19 steroids, namely 11β-hydroxyandrostenedione. Our data provide evidence that increase in gene expression for steroidogenic enzyme mRNAs may not totally be reflected by an enhancement of enzymatic activity, thus suggesting that ACTH may have post-transcriptional effects. Furthermore, steroid themselves may be involved in the regulation of C19 steroid production, particularly by an interaction with *P*450c21 and *P*450c17.

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