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The Effect of Prolactin, Human Chorionic Gonadotropin, Insulin and Insulin-like Growth Factor 1 on Adrenal Steroidogenesis in Isolated Guinea-pig Adrenal Cells

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The controlling mechanism for adrenal androgen production has not been elucidated. The presence of receptors for prolactin, human chorionic gonadotropin (hCG), insulin and insulin-like growth factor 1 (IGF-1) in the adrenal cortex raises the possibility of their involvement in the control of adrenal steroidogenesis. This study was undertaken to investigate the effects of prolactin, hCG, insulin and IGF-1 in the presence and absence of ACTH on cortisol and androgen production using isolated guinea-pig adrenal cells. hCG 10^{-7} and 10^{-6} M significantly increased cortisol ($P < 0.05$) production. hCG 10^{-6} M significantly increased androstenedione (A4) ($P < 0.05$) production. In the presence of ACTH, 10^{-12} M, hCG 10^{-6} M significantly increased the cortisol ($P < 0.01$) and A4 ($P < 0.01$) responses. Although the mean cortisol and A4 response to ACTH 10^{-9} M was reduced in the presence of hCG 10^{-6} M, this was not statistically significant. Prolactin 10^{-8} M increased cortisol ($P < 0.01$), A4, and dehydroepiandrosterone ($P < 0.05$) production. In the presence of ACTH 10^{-12} M, prolactin 10^{-8} M increased the cortisol and A4 ($P < 0.05$) responses. However, the maximally ACTH-stimulated cortisol and A4 responses were not significantly altered in the presence of prolactin 10^{-8} M. Insulin 10^{-11}–10^{-8} M and IGF-1 10^{-10}–10^{-7} M resulted in no significant increase in cortisol, A4 or dehydroepiandrosterone production. This study suggests that prolactin and hCG could play a role in modulation of adrenal steroidogenesis, particularly when ACTH levels are low. However, there was no evidence that prolactin or hCG is the specific cortical androgen stimulating hormone.

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INTRODUCTION

The precise regulation of adrenal androgen production remains controversial [1]. ACTH is known to be the principal modulator of glucocorticoid production and is also involved in adrenal androgen production [2]. However, it is likely that other factors are also involved in the control of adrenal androgen production [1]. A role for prolactin in adrenal steroidogenesis has been postulated based on receptor presence in adrenal tissue [3]. Studies have shown correlation between high prolactin levels and adrenal androgens in blood, particularly dehydroepiandrosterone-sulphate (DHEAS), [4, 5]. Human chorionic gonadotropin (hCG) has been reported to increase DHEAS production in human foetal adrenal tissue [6]. Receptors for insulin and insulin-like growth factor 1 (IGF-1) have been identified in bovine [7] and human [8] adrenal glands.

The presence of receptors for prolactin, hCG, insulin and IGF-1 prompts the question whether these peptides have a role in modulating adrenal steroidogenesis. In the absence of suitable human adrenal tissue, the guinea-pig adrenal cortex with its functional, biochemical and morphological similarities to the human gland [9–11] was considered to be a suitable model for investigation of factors controlling adrenal steroidogenesis. In the present study we investigated the effect of prolactin, hCG, insulin and IGF-1 on basal and ACTH-stimulated androgen and cortisol production, using an isolated guinea-pig adrenal cell system.

METHODS

Cell suspension preparation

Adrenal cell suspensions were prepared using adrenals from male Dunkan–Hartley guinea-pigs

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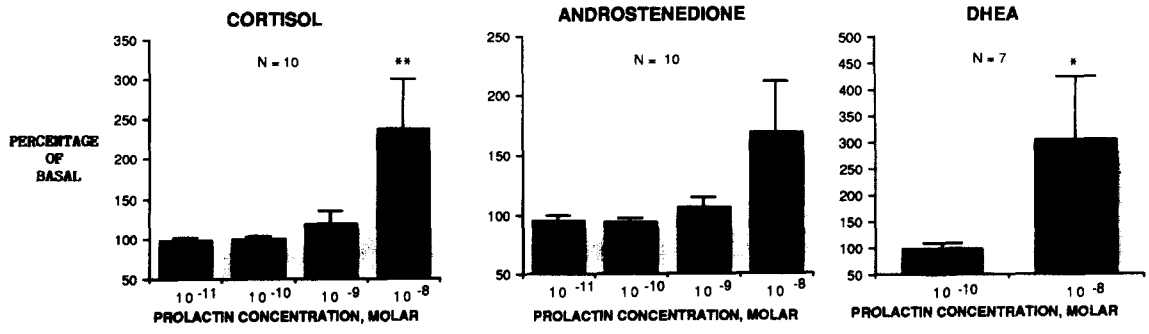

Fig. 1. The cortisol, androstenedione and DHEA responses to prolactin (10^{-11} to 10^{-8} M) in isolated guinea-pig adrenal cells incubated for 2 h at 37°C. DHEA was measured in trilostane (6 μM)-treated cells. Values are expressed as percentage change relative to basal production, mean + SE, where basal values were as follows: cortisol 68.3 ± 12.6 pmol/10^5 cells, $n = 10$; androstenedione 6.7 ± 1.2 pmol/10^5 cells, $n = 10$; DHEA 1.7 ± 0.3 pmol/10^5 cells, $n = 7$. Significant increases as compared with basal production: *$P < 0.05$; **$P < 0.01$.

(800–1000 g) which had been pretreated with long-acting ACTH (Acthar Gel, Rorer, Pharmaceuticals, Eastbourne, England; 0.2 ml equivalent to 8 IU ACTH, s.c. 3 days prior to adrenalectomy. A detailed account of the protocol has been outlined elsewhere [11]. In brief, the chopped adrenal tissue was incubated in a collagenase solution (Collagenase Type I from clostridium histolyticum, Sigma Chemical Co., St Louis, U.S.A., Cat No. CO130, 2 mg/ml) for 20 min at 37°C. Following centrifugation (400 *g* for 15 min), the tissue pellet was dispersed by multiple passage through teflon-tubing attached to a syringe. Isolated adrenal cells were harvested after washing and centrifugation before final resuspension in Eagle's Modified Essential Medium (EMEM, Flow Labs, Scotland, Cat. No. 10-105-22) containing calcium chloride (B.D.H., Poole, England), 8 mM and sodium ascorbate (Sigma, Cat. No. A7906), 1 mM.

Cells were incubated in the presence of prolactin 10^{-11}–10^{-8} M, hCG 10^{-9}–10^{-6} M, insulin 10^{-11}–10^{-8} M and IGF-1 10^{-10}–10^{-7} M. The highest concentration of each peptide was also incubated in the presence of ACTH 10^{-12} M and ACTH 10^{-9} M. In those tubes intended for DHEA measurement, trilostane, an inhibitor of 3β-ol dehydrogenase, Δ4-5 isomerase (kindly donated by Sterling Winthrop, England), was included at a final concentration of 6 μM. This increased the yield of DHEA but did not qualitatively change DHEA production. Experimental agents were added in 100 μl volumes to plastic culture tubes. Following addition of cell suspension (800 μl) the final tube volume was adjusted to 1 ml with resuspending medium where necessary. Cells were incubated for 2 h at 37°C under an atmosphere of 100% oxygen and then frozen.

Steroid measurement

Following the release of the intracellular steroid content into the incubation medium by repeated freezing and thawing, the steroids cortisol, androstenedione and DHEA were measured by specific radioimmunoassays. Androstenedione and DHEA were measured following extraction into diethyl ether and chromatographic purification over celite columns. Cortisol was measured directly without prior extraction or chromatographic purification. Further details of the assay procedures, together with information regarding the antisera, labelled ligands and the specificities, sensitivities and imprecision of the assays have been reported previously [11].

Source of peptide hormones

Prolactin purified from human pituitaries (3rd IS 1988, 84/500; 1 ampoule containing 0.053 IU

Table 1. Effect of prolactin on ACTH-stimulated steroidogenesis[a]

	Cortisol		Androstenedione	
	Mean ± SE(%)	*n*	Mean ± SE(%)	*n*
Basal	100 ± 18	9	100 ± 18	9
Prolactin 10^{-8} M	230 ± 69	9	171 ± 47	9
ACTH 10^{-12} M	210 ± 54	9	158 ± 27	9
Prolactin + ACTH 10^{-12} M	376 ± 105	9	**253 ± 43	9
ACTH 10^{-9} M	1391 ± 185	8	824 ± 128	8
Prolactin + ACTH 10^{-9} M	1531 ± 209	8	787 ± 99	8

[a]Values are expressed as percentages of basal, where basal values were as follows (mean ± SE); Cortisol 68.3 ± 12.6 pmol/10^5 cells; androstenedione 6.7 ± 1.2 pmol/10^5 cells.

**Significantly greater than values obtained with ACTH 10^{-12} M alone, $P < 0.05$.

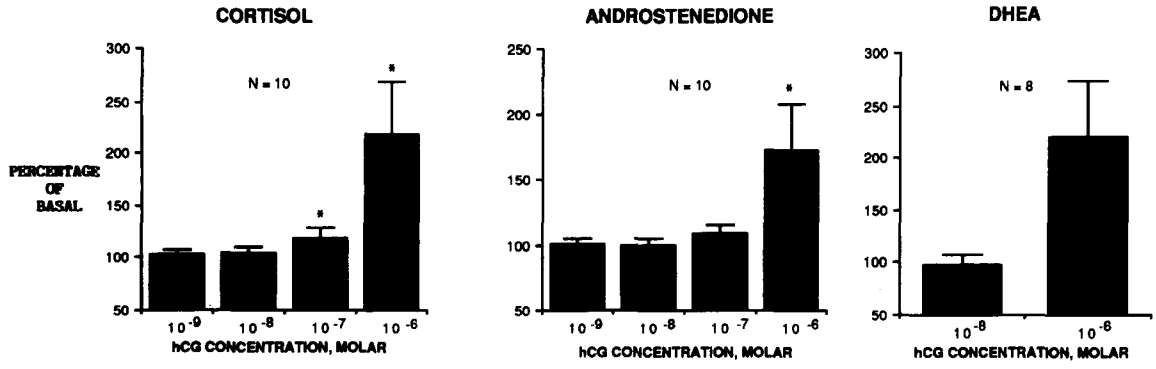

Fig. 2. The cortisol, androstenedione and DHEA responses to hCG (10^{-9} to 10^{-6} M) in isolated guinea-pig adrenal cells incubated for 2 h at 37°C. DHEA was measured in trilostane (6 μM)-treated cells. Values are expressed as percentage change relative to basal production, mean + SE, where basal values were as follows: cortisol 61.0 ± 13.2 pmol/10^5 cells, $n = 10$; androstenedione 6.1 ± 1.1 pmol/10^5 cells, $n = 10$; DHEA 2.1 ± 0.3 pmol/10^5 cells, $n = 8$. Significant increases as compared with basal production: *$P < 0.05$.

equivalent to 3 μg human prolactin) was obtained from the National Institute of Biological Standards and Controls (N.I.B.S.C., Potters Bar, England). Human insulin (1st IS , 1986, 83/500; 1 ampoule containing 26.0 IU/mg) was also obtained from N.I.B.S.C. Human IGF-1, purified recombinant material, was obtained from Peninsula Labs (Merseyside, England, Cat. No. IP9010) and hCG, purified from urine of pregnant women, was kindly donated by Paines and Byrne, (Greenford, Middlesex, England, Code No. G05053). Synthetic $ACTH_{1-24}$ was kindly donated by Ciba-Geigy (U.K.). The hCG and prolactin preparations (10^{-5} and 10^{-6} M, respectively) contained no detectable ACTH when submitted to a sensitive ACTH radioimmunoassay (Double Antibody Method, Diagnostic Products Corporation, Los Angeles, U.S.A.).

Statistics

Each experimental condition was set up in duplicate on 7–10 occasions. The Wilcoxin Ranked Sum for non-parametric data was used to evaluate the significance of alterations in steroid production [12].

RESULTS

Prolactin was studied over the concentration range 10^{-11}–10^{-8} M (see Fig. 1). Cortisol, androstenedione and DHEA production was increased in the presence of prolactin 10^{-8} M, reaching statistical significance for both the cortisol ($P < 0.01$) and the DHEA response ($P < 0.05$). The effect of prolactin 10^{-8} M on ACTH-stimulated steroid production is shown in Table 1. In the presence of a low concentration of ACTH (10^{-12} M), prolactin 10^{-8} M increased the cortisol and andreostenedione responses, reaching significance in the case of androstenedione $P < 0.05$. However, the maximally ACTH-stimulated cortisol and androstenedione responses were not significantly altered in the presence of prolactin 10^{-8} M.

hCG was studied over the concentration range 10^{-9}–10^{-6} M (see Fig. 2). Steroid production was increased in the presence of hCG, reaching statistical significance in the presence of hCG 10^{-7} and 10^{-6} M for cortisol ($P < 0.05$), and hCG 10^{-6} M for androstenedione ($P < 0.05$). hCG 10^{-6} M significantly increased the cortisol ($P < 0.01$) and androstenedione

Table 2. Effect of hCG on ACTH-stimulated steroidogenesis[a]

	Cortisol		Androstenedione	
	Mean ± SE(%)	*n*	Mean ± SE(%)	*n*
Basal	100 ± 22	9	100 ± 18	9
hCG 10^{-6} M	201 ± 55	9	172 ± 39	9
ACTH 10^{-12} M	237 ± 57	8	174 ± 28	9
hCG + ACTH 10^{-12} M	**410 ± 94	8	**266 ± 48	9
ACTH 10^{-9} M	1867 ± 270	9	1019 ± 114	9
hCG + ACTH 10^{-9} M	1637 ± 211	9	894 ± 84	9

[a]Values are expressed as percentage of basal, where basal values were as follows (mean ± SE); cortisol 61.0 ± 13.2 pmol/10^5 cells; androstenedione 6.1 ± 1.1 pmol/10^5 cells.

**Significantly greater than the values obtained with ACTH 10^{-12} M alone, $P < 0.01$.

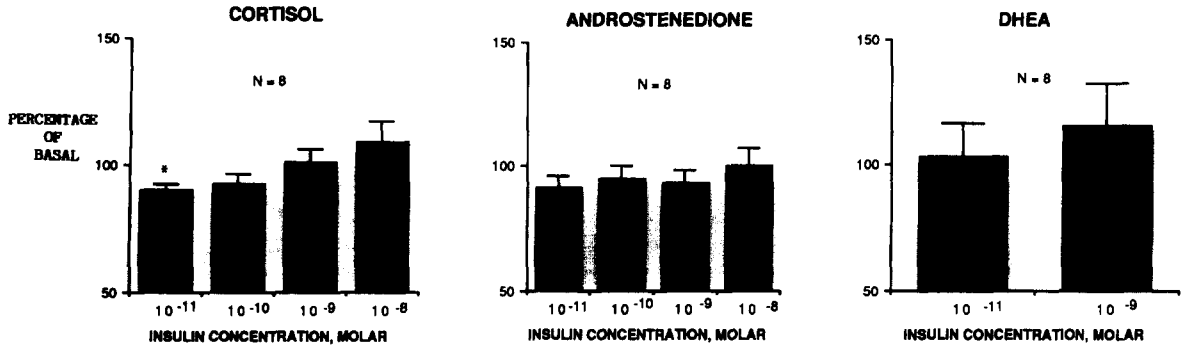

Fig. 3. The cortisol, androstenedione and DHEA responses to insulin (10^{-11} to 10^{-8} M) in isolated guinea-pig adrenal cells incubated for 2 h at 37°C. DHEA was measured in trilostane (6 μM)-treated cells. Values are expressed as percentage change relative to basal production, mean + SE, where basal values were as follows: cortisol 70.1 ± 14.8 pmol/10^5 cells, $n = 8$; androstenedione 6.6 ± 1.1 pmol/10^5 cells, $n = 8$; DHEA 1.5 ± 0.3 pmol/10^5, $n = 8$. Significant inhibition as compared with basal production: $^*P < 0.05$.

($P < 0.01$) responses to ACTH 10^{-12} M stimulation (Table 2). The summation of the responses to hCG 10^{-6} M and ACTH 10^{-12} M alone approximately equalled the response to the two peptides combined for cortisol and androstenedione. hCG 10^{-6} M reduced the mean cortisol and androstenedione response to ACTH 10^{-9} M, but this reduction did not reach statistical significance.

The presence of insulin 10^{-11}–10^{-8} M did not result in a significant increase in cortisol, androstenedione or DHEA production in isolated guinea-pig adrenal cells during a 2 h incubation period (Fig. 3). In fact there was a slight but a statistically significant inhibition of cortisol production in the presence of insulin 10^{-11} M. The mean cortisol and androstenedione responses to ACTH 10^{-9} M were 32% higher when insulin 10^{-9} M was also present but this increase did not reach statistical significance. IGF-1 was studied over the concentration range 10^{-10}–10^{-7} M (see Fig. 4). IGF-1 had no significant effect on cortisol, androstenedione or DHEA production.

DISCUSSION

The guinea-pig adrenal cortex bears a number of similarities to the human adrenal gland which make it an acceptable model for investigation of adrenal steroidogenesis [9–11]. The guinea-pig adrenal cortex has similar zonation to the human gland, with a particularly well developed zona reticularis, an active 17-hydroxylase system and a requirement for ascorbic acid. Cortisol is the major glucocorticoid product of the guinea-pig adrenal cortex, which can also synthesize considerable amounts of androstenedione and smaller amounts of DHEA. The measurement of DHEA was facilitated by the inclusion of the 3β-ol dehydrogenase, Δ4-5 isomerase inhibitor, trilostane. Under these circumstances, measurement of DHEA provides an index of the activity of the Δ5 androgen synthetic pathway. Guinea-pig adrenal steroidogenesis is also responsive to ACTH stimulation [9–11]. In this study we used an optimized guinea-pig adrenal cell preparation [11] to examine the effects of prolactin, hCG, insulin and IGF-1 on adrenal steroidogenesis.

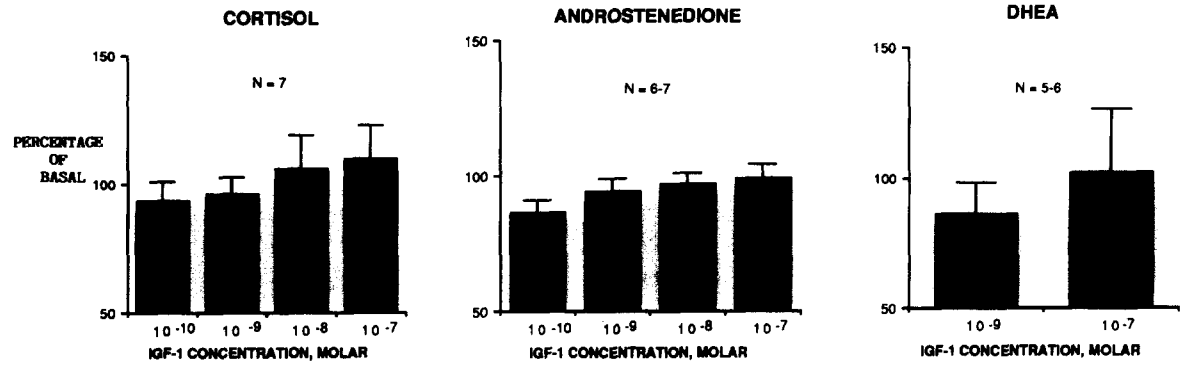

Fig. 4. The cortisol, androstenedione and DHEA responses to IGF-1 (10^{-10} to 10^{-7} M) in isolated guinea-pig adrenal cells incubated for 2 h at 37°C. DHEA was measured in trilostane (6 μM)-treated cells. Values are expressed as percentage change relative to basal production, mean + SE, where basal values were as follows: cortisol 74.1 ± 15.5 pmol/10^5 cells, $n = 7$; androstenedione 8.2 ± 1.4 pmol/10^5 cells, $n = 7$; DHEA 1.6 ± 0.2 pmol/10^5 cells.

In the present study prolactin was shown to stimulate steroidogenesis, but there was no preferential stimulation of androgen rather than glucocorticoid production. Previous studies using prolactin produced conflicting results. O'Hare *et al.* [13], reported no alteration in steroid production in the presence of prolactin in either dispersed zona fasciculata or zona reticularis cells or in long-term cultured adrenal cells. Provencher *et al.* [14], reported that prolactin had no effect on basal steroid production, but resulted in a net increase in C19 steroid production in ACTH 10^{-8} M-stimulated cultured guinea-pig adrenal cells, with a concomitant decrease in glucocorticoid production. In an *in vivo* study with baboon foetuses, Pepe *et al.* [15], showed that injection of prolactin resulted in a significant increase in DHEA but not cortisol secretion. Although increased androgen levels have been reported in hyperprolactinaemic patients [3, 16], the present study does not support a role for prolactin as the specific adrenal androgen stimulating hormone. However, the effects of prolactin on adrenal steroidogenesis may be important when ACTH secretion is low or in hyperprolactinaemic states.

In the present study hCG 10^{-6} M significantly stimulated cortisol and androstenedione production. Previous studies using human foetal adrenal tissue reported an increase in DHEAS production in the presence of hCG [6, 17]. However, other groups reported a lack of effect of hCG on adrenal steroidogenesis [18, 19.]. In this study hCG stimulated steroidogenesis at concentrations which are found in maternal plasma in pregnancy. During pregnancy plasma and urinary free cortisol are increased and there is a reduction in the diurnal rhythm of plasma free cortisol [20]. This could be explained by increased hCG secretion which would increase cortisol production at times when ACTH levels were low. Increased cortisol production during pregnancy may be protective to the foetus since in the foetal adrenal 3β-hydroxysteroid dehydrogenase activity is less than occurs following birth and this may limit *de novo* cortisol synthesis throughout much of the pregnancy [21].

In order to rule out the possibility that stimulation of adrenal steroidogenesis by prolactin and hCG might be due to contamination of the highly purified peptides by minute amounts of ACTH, high concentrations of hCG (10^{-5} M) and prolactin (10^{-6} M) were submitted to radioimmunoassay using an ACTH R.I.A. from Diagnostic Products. These concentrations represent 10 and 100 times, respectively, the highest concentrations used in the cell suspension experiments. Neither peptide contained any detectable ACTH (<12 pg/ml). Therefore, the concentration of ACTH, if present at all in the peptide preparations, was $<3 \times 10^{-13}$ M in the hCG preparation and $<3 \times 10^{-14}$ M in the prolactin preparation at the highest concentrations studied in cell suspensions. Since stimulation of steroidogenesis by prolactin 10^{-8} M and hCG 10^{-6} M was of the same order as that achieved by ACTH 10^{-12} M, therefore ACTH contamination could not account for the stimulatory effects of these peptides on steroidogenesis.

Insulin and IGF-1 had no significant stimulatory effects on adrenal steroidogenesis in isolated guinea-pig adrenal cells. Penhoat *et al.* [7] reported that bovine adrenal cells cultured in the presence of IGF-1 or insulin had enhanced capacity to produce pregnenolone and increased activity of several steroid enzymes. Kramer *et al.* [22], also using cultured bovine adrenal cells reported that insulin directly affected ACTH-stimulated steroidogenesis. Although the results from the present study do not support a significant role for insulin and IGF-1 in acute stimulation of adrenal steroidogenesis, it is possible that these two polypeptides may play a more long-term role in the modulation of steroidogeneis.

Pro-opiomelanocortin-derived peptides, particularly the "Joining Peptide" (JP), have been implicated in the control of human adrenal androgen secretion [23] but this has not been confirmed by others [24]. In a previous study using the guinea-pig adrenal cell preparation we reported that the C-terminal portion of pro-opiomelanocortin, β-lipotrophin, stimulated cortisol and androgen production but we found no consistent effect of JP on adrenal steroidogenesis [11]. In the present study, we have demonstrated that prolactin and hCG at levels found in mild hyperprolactinemia or in pregnancy, respectively, stimulate cortisol and androgen production during short-term incubation of isolated guinea-pig adrenal cells. These hormones could play a role in modulation of adrenal steroidogenesis particularly in circumstances where ACTH levels are low. However, it is unlikely that either of these polypeptides is the specific cortical androgen stimulating hormone since no preference for stimulating androgen rather than cortisol production was demonstrated.

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