Catecholamines inhibit steroidogenesis by cultured porcine thecal cells

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The ovaries of many species contain catecholamines and β-adrenergic receptors. The present studies were done to determine if catecholamines play a role in the regulation of androgen production by porcine theca cells. Basal and luteinizing hormone (LH)-stimulated androstenedione production was significantly inhibited by noradrenaline and isoproterenol. The inhibitory effects were dose-dependent and were enhanced when the cultures contained the carboxy-*O*-methyl transferase inhibitor, U-0521. The inhibitory effect of isoproterenol was reversed by the β-adrenergic antagonist, metoprolol. Isoproterenol caused a generalized inhibition of LH-stimulated steroidogenesis, decreasing the accumulation of pregnenolone, progesterone, androstenedione and estradiol in the culture medium. These studies suggest that catecholamines may be important regulators of thecal androgen production.

Porcine thecal cell; Steroidogenesis; Catecholamine

1. INTRODUCTION

The ovaries of many species contain catecholamines [1,2]. There is little information on the distribution of catecholamines within the mammalian ovary, but, in the hen, most of the ovarian noradrenaline is found in the theca layer [3]. Immature and adult rat ovaries contain β_2-adrenergic receptors [4-9]. In the rat, catecholamine receptors are found in the ovarian granulosa [5,6], luteal [4] and theca-interstitial cells [9]. Many studies have shown β-receptor-mediated effects of catecholamines on ovarian progesterone production [6,7,10-12]. Catecholamines also act on cultured rat theca-interstitial cells to increase gonadotropin-stimulated androgen production [9,13]. The objective of the present studies was to examine the effects of catecholamines on androgen production by dispersed porcine thecal cells.

2. MATERIALS AND METHODS

Thecal cells were obtained from the ovaries of prepubertal gilts and cultured as described previously [14]. Effects of catecholamines were studied in the absence and presence of a maximally stimulating dose of LH (250 ng/ml).

LH used in these studies was USDA bovine LH-B5. The catechol-*O*-methyl transferase (COMT) inhibitor U-0521 (propiophenone) was obtained from The Upjohn Co. (Kalamazoo, MI). Clonidine, isoproterenol and noradrenaline were obtained from Sigma Chemical Co. (St. Louis, MO). Metoprolol was obtained from CIBA-Geigy (Dorval, PQ).

Following culture, media were removed and stored at -20°C until assayed for androstenedione [15], progesterone [16], pregnenolone [14] and estradiol [17] by radioimmunoassay.

Statistical comparisons were made by analysis of variance. When significant effects were observed, Duncan's new multiple range test was used for multiple comparisons. Treatments were replicated in quadruplicate within an experiment, and each experiment was performed 2-3 times.

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3. RESULTS

Androstenedione production by LH-stimulated (250 ng/ml) porcine thecal cells cultured in the presence of the β-adrenergic agonist isoproterenol (5×10^{-9}–10^{-5} M) or noradrenaline (10^{-8}–10^{-4}M) was significantly ($P < 0.05$) inhibited in a dose-dependent manner (Fig. 1A,B). Neither isoproterenol nor noradrenaline interfered in the androstenedione radioimmunoassay. Addition of the α-adrenergic agonist clonidine (10^{-7}–10^{-4} M) to cultured thecal cells did not significantly effect basal or LH-stimulated androstenedione production (Table I).

Catecholamines undergo both non-enzymatic oxidation and enzymatic degradation by monoamine oxidase and COMT. In the presence of U-0521 (10 μM), an inhibitor of the COMT enzyme, the effects of isoproterenol on thecal androstenedione production were significantly ($P < 0.05$) enhanced so that concentrations of isoproterenol as low as 10^{-8} M caused significant ($P < 0.05$) inhibitory effects on androgen production (Fig. 2). At high concentrations of isoproterenol (10^{-5} or 10^{-4} M) there was no significant effect of the addition of U-0521.

The β-adrenergic antagonist metoprolol (10^{-4} M) was used to determine if the inhibitory effects were mediated via β-adrenergic receptors. Isoproterenol was added to the cultures 30 min after the addition of metoprolol to allow binding of the antagonist to

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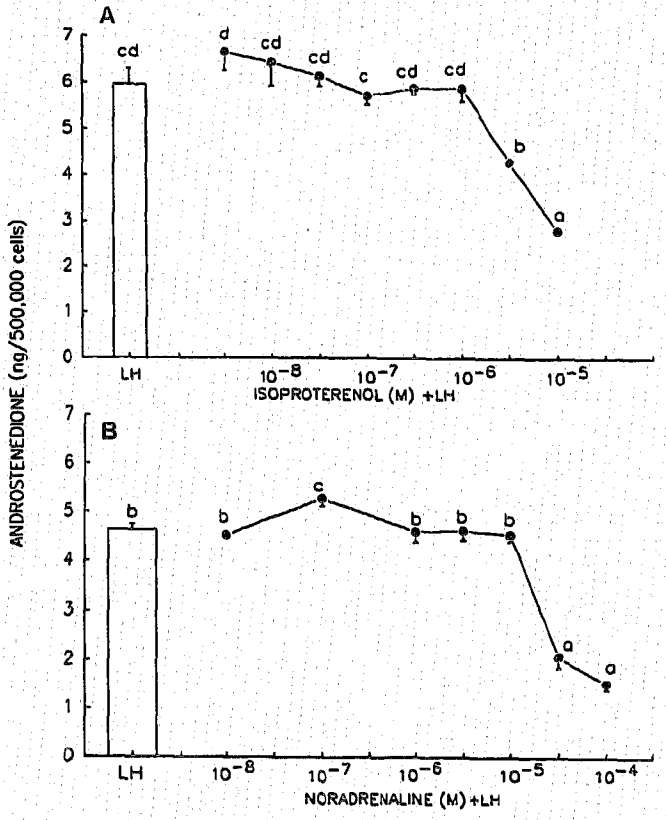

Fig. 1. Inhibition of LH-stimulated thecal androstenedione production by isoproterenol (A) and noradrenaline (B). LH-stimulated thecal cells were cultured in the presence of noradrenaline or isoproterenol. Data are the mean ± SE of quadruplicate cultures from a typical experiment which was replicated three times. Where no error bar is shown the SE is within the limit of the symbol. Values with different superscripts are significantly ($P<0.05$) different.

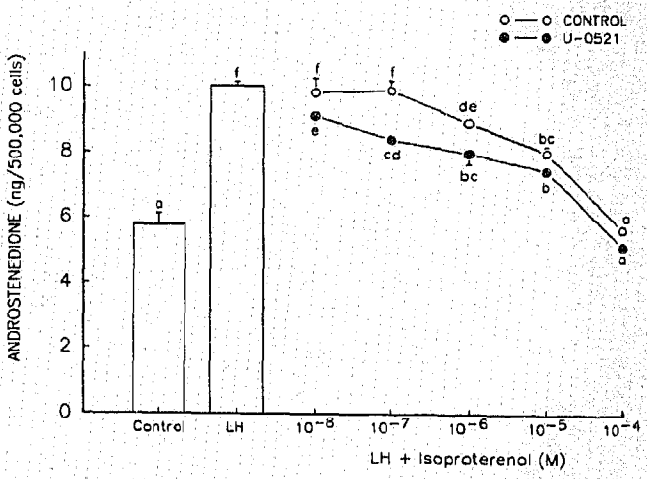

Fig. 2. Effect of the catechol-*O*-methyl transferase (COMT) inhibitor U-0521 on the inhibition of thecal androstenedione production by isoproterenol. Thecal cells were incubated in the absence (Control) and presence of LH and isoproterenol, with or without U-0521. Values represent the mean ± SE of quadruplicate cultures in a typical experiment which was replicated twice. Where no error bar is shown the SE is within the limit of the symbol. Values with different superscripts are significantly ($P<0.05$) different.

catecholamine receptors. Metoprolol reversed the isoproterenol inhibition of LH-stimulated androstenedione production (Table II).

Investigation of the site(s) in the steroidogenic pathway at which isoproterenol exerted its inhibitory effect showed that treatment with isoproterenol decreased the production of all steroids by both basal and LH-stimulated thecal cell cultures (Table III).

4. DISCUSSION

These studies have shown that β-adrenergic agents inhibit porcine thecal cell androstenedione production. Each experiment began with a unique population of theca cells, and there was variability in the responses of the cells between experiments, due to unknown causes. Althought similar trends were observed in replicate experiments, the absolute values of steroid production varied among experiments. It is not known if the stage of ovarian development affects the thecal cells' responsiveness to catecholamines.

The inhibitory effect of catecholamines on androgen production was reversed by the β-receptor antagonist

Table I

Effect of clonidine on theca cell androstenedione accumulation

	Androstenedione (ng/500 000 cells)[a]	
Clonidine (M)	Control	LH
-	11.87 ± 0.55*	25.63 ± 2.37**
10^{-7}	10.35 ± 1.05*	25.69 ± 0.54**
10^{-6}	11.77 ± 0.44*	25.36 ± 1.08**
10^{-5}	10.01 ± 0.42*	25.96 ± 2.22**
10^{-4}	10.46 ± 0.42*	24.04 ± 0.15**

[a]Theca cells were cultured in the absence (Control) and presence of LH (250 ng/ml) with or without increasing concentrations of the α-adrenergic agonist clonidine (10^{-7}–10^{-4} M). Data are the mean ± SE of quadruplicate cultures from a typical experiment which was replicated twice. Values with different superscripts are significantly ($P<0.05$) different

Table II

Effect of metoprolol on the inhibition of thecal androstenedione production by isoproterenol

	Androstenedione (ng/500 000 cells)	
Treatment[a]	—	Metoprolol
CON	3.80 ± 0.20*	4.31 ± 0.37*,**
ISO	2.89 ± 0.28*	4.07 ± 0.64*,**
LH	7.16 ± 0.62***	7.11 ± 0.58***
LH + ISO	5.58 ± 0.26**	7.23 ± 0.45***

[a]Theca cells were cultured in the absence (CON) and presence of LH (250 ng/ml) with or without isoproterenol (ISO; 10^{-5} M). Some cultures contained the β-adrenergic antagonist metoprolol (10^{-4} M). Data are the mean ± SE of quadruplicate cultures from a typical experiment which was replicated twice. Values with different superscripts are significantly ($P<0.05$) different

Table III

Isoproterenol inhibition of thecal androstenedione production

Steroid[a]	Steroid accumulation (ng/500 000 cells)			
	CON	ISO	LH	LH + ISO
P_5	0.40 ± 0.19*	0 ± 0*	1.49 ± 0.30**	0.45 ± 0.08*
P_4	3.75 ± 0.28***	1.56 ± 0.09*	8.56 ± 0.30****	2.82 ± 0.20**
Adione	10.34 ± 0.32**	6.10 ± 0.20*	30.99 ± 2.43****	20.42 ± 0.93***
E_2	0.99 ± 0.07**	0.44 ± 0.07*	2.60 ± 0.20***	1.08 ± 0.02**

[a]Theca cells were cultured with (LH) or without (CON) LH (250 ng/ml) in the absence or presence of isoproterenol (ISO; 10^{-4} M). At the end of 48 h of culture the medium was removed for RIA of pregnenolone (P_5), progesterone (P_4), androstenedione (Adione) and estradiol (E_2). Values represent the mean ± SE of quadruplicate cultures from a typical experiment which was replicated twice. Values with different superscripts are significantly ($P<0.05$) different within each steroid

metoprolol. Although the β-adrenergic receptors in rat theca-interstitial cells have been characterized [9], there have been no reports of catecholamine receptors in porcine thecal cells. The observation that catecholamines inhibit porcine thecal androgen production is in contrast to the effect of catecholamines on rat theca-interstitial cells. Other than the obvious species differences, no explanation for these divergent results is apparent. In both species, however, the effects on steroidogenesis appear to be mediated at β-, but not α-adrenergic receptors [9,13].

The concentrations of catecholamines required to elicit significant effects were very high. The concentration of noradrenaline in follicular fluid from small porcine preovulatory follicles is 34 nM [8], which is 10 times higher than that in the general circulation, but is still too low to inhibit thecal androstenedione production in our cultures. High doses of catecholamines may be required since they were added at the time of plating, and presumably were metabolized rapidly during the culture period. The addition of the COMT inhibitor, U-0521, significantly increased the inhibitory effect of isoproterenol. This supports a previous report that the porcine ovarian follicular wall has an active COMT enzyme system [18] and may account for the high catecholamine concentrations required to observe inhibitory effects in culture.

Catecholamines caused a generalized suppression of theca cell steroidogenesis, but it is not known whether this was due to a specific inhibition of each steroidogenic enzyme or the suppression of an early step in the steroidogenic pathway.

If the catecholamine inhibition of thecal androstenedione production is of physiological significance, there must be a way to supply the thecal cells with catecholamines. The most likely sources of noradrenaline would be the terminals of postganglionic sympathetic neurons which innervate thecal cells of all sizes of developing follicles [1], or adrenal catecholamines which could reach the thecal cells by their abundant blood supply. There may also be cells within the ovary capable of noradrenaline production, but there is no direct evidence for their existence. The fact that catecholamines and β-adrenergic receptors are present in the ovary suggest that catecholamines may be important intra-ovarian regulators of thecal cell activity.

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