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DISCORDANT REGULATION BY LUTEINIZING HORMONE OF ORNITHINE DECARBOXYLASE ACTIVITY AND TESTOSTERONE PRODUCTION IN ISOLATED RAT TESTICULAR CELLS IN VITRO

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SUMMARY: Possible functional relationship between luteinizing hormone-stimulated ornithine decarboxylase and testosterone production was examined in rat testicular interstitial cells in vitro. Although luteinizing hormone enhanced both ornithine decarboxylase activity and testosterone production at a similar physiological dose range, we found dissociation in the two responses in terms of their temporal aspect and the way they were affected by an irreversible inhibitor of ornithine decarboxylase, alpha-difluoromethylornithine, and protein synthesis inhibitor cycloheximide. The results suggest that there appears to be no causal coupling between luteinizing hormone-stimulated enzyme activity and testicular steroidogenesis.

Cellular responses to various hormones and growth-promoting agents are associated with marked enhancement of the activity of polyamine biosynthetic enzymes and polyamine accumulation (1-3). However, the functional coupling of increased polyamine levels with specific cellular response(s) is less well understood.

We have recently demonstrated a specific and dose-dependent stimulation by LH of testicular interstitial cell and purified Leydig cell ornithine decarboxylase activity in vitro, the rate-limiting enzyme of polyamine biosynthesis (4). This stimulation occurs with physiological doses of LH that also augment testicular steroidogenesis. The present studies examine possible functional relationship between LH-stimulated ornithine decarboxylase activity and testosterone production by the same cells in vitro.

Abbreviations Used: DFMO; Alpha-difluoromethylornithine; LH; Luteinizing Hormone

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MATERIALS AND METHODS

Chemicals: Ovine LH (NIH-LH-S23) was obtained from the Hormone Distribution Office (NIAMMD, NIH, Bethesda, Maryland). Collagenase (Type I), 3-isobutyl-1-methyl-xanthine and cycloheximide were purchased from Sigma Chemical Company, St. Louis, Missouri. L-[1-^{14}C] Ornithine monohydrochloride (sp. radioactivity 58.9 mCi/mmol) was obtained from New England Nuclear Corporation, Boston, Massachussets. Culture Medium 199 with Hank's salts and 25 mM Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer (pH 7.4) was from Gibco, Grand Island, New York. DFMO was generously provided by Dr. D.J. Wilkins, Centre dRecherche Merrell International, Strasbourg Cedex, France.

Animals and Tissue: Male Sprague-Dawley rats (60-120 days old) were purchased from Zivic-Miller Laboratories, Inc., Allison Park, Pennsylvania. Testes were quickly removed from decapitated rats, decapsulated and kept on ice. Testicular interstitial cells were prepared by collagenase digestion as previously described (5). The incubation with collagenase was carried out for 15 min.

Culture Conditions: Interstitial cells (1.5-2.5 x 10^7 viable cells, determined by Trypan-Blue exclusion) were incubated in 10 ml of Medium 199 containing 1.0 mg/ml bovine serum albumin and 25 mM Hepes buffer, pH 7.4 (culture medium) for 1-4 h at 34°C in an atmosphere of O_2/CO_2 (95:5, v:v). LH, in concentrations as indicated, 0.1 mM isobutyl-methylxanthine (dissolved in dimethyl sulfoxide and added in the total volume of 0.5 µl/ml culture medium), cycloheximide and DFMO were added at the beginning of the incubation. Control culture received a corresponding volume of the solvent. Following incubation cells were recovered by centrifugation (at 600 x g), washed twice in homogenizing medium containing 0.25 M sucrose and then homogenized in 2.5 ml of ice-cold 25 mM Tris/HCl buffer, pH 7.4, containing 0.1 mM EDTA and 5.0 mM dithiothreitol. The homogenates were centrifuged for 30 min at 20,000 x g and supernatants were used for the assay of ornithine decarboxylase activity and protein concentration.

Enzyme Assay: Ornithine decarboxylase activity was determined by measuring in duplicate the liberation of $^{14}CO_2$ from L-[1-^{14}C] ornithine under conditions as previously described (6). The enzymatic activity was expressed as pmol of CO_2 released/mg cytosolic protein per 30 min. Cytosolic protein concentration was determined by the method of Lowry et al (7) as modified by Munro and Fleck (8), with bovine serum albumin as the standard.

Testosterone Radioimmunoassay: Testosterone was measured by radioimmunoassay on ether-extracted aliquots of incubation medium without prior chromatography, following procedures which have been described previously (5). Results are expressed as ng/10^6 cells per time of incubation (usually 4 h).

Results were analyzed by Student's t test.

RESULTS

We have recently reported a specific and dose-dependent stimulation by LH of rat testicular interstitial cell ornithine decarboxylase *in vitro* (4). The minimally effective dose of LH was 10 pg/ml and the maximal stimulation of the enzyme activity, which varied between 40-100% above the respective control in several independent experiments, was achieved

TABLE I

LH-Stimulated Testosterone Production

Treatment	Testosterone (ng/10^6 cells per 4 h)*	p**
Control	3.14 ± 0.03	
LH 1.0 pg/ml	3.23 ± 0.06	
10.0 pg/ml	6.41 ± 0.14	<0.001
100.0 pg/ml	11.83 ± 1.05	<0.001
1.0 ng/ml	11.89 ± 0.15	<0.001
100.0 ng/ml	12.36 ± 0.20	<0.001

*Results are means ± S.E.M. of 3 incubations
** vs control

with LH dose of 100 pg/ml at 4 h of incubation (4). Testosterone production by interstitial cells as shown in Table I followed a similar dose response, although the magnitude of this response (about 4-fold stimulation) was much higher.

To examine the relationship between the activity of ornithine decarboxylase and testosterone production, we employed an enzyme-activated irreversible inhibitor of ornithine decarboxylase, DFMO, which causes marked depletion of cellular polyamines in several systems (9). As shown in Table II, DFMO caused a dose-dependent inhibition of LH-stimulated ornithine decarboxylase activity but produced no statistically significant inhibition (10-12%) of testosterone production.

TABLE II

Effect of DFMO on LH-stimulated Ornithine Decarboxylase Activity and Testosterone Production

Treatment		Ornithine Decarboxylase Activity (pmol/mg protein per 30 min)	Testosterone (ng/10^6 cells per 4 h)
LH (ng/ml)	DFMO (mM)		
0	0	37.86 ± 2.67	1.22 ± 0.02
100	0	53.81 ± 2.12*	4.17 ± 0.15***
100	0.1	30.58 ± 1.82**	4.21 ± 0.13***
100	1.0	22.97 ± 2.63**	3.74 ± 0.15***
100	10.0	22.18 ± 1.36**	3.69 ± 0.10***

Results are means ± S.E.M. of 3 incubations. LH and DFMO were added in the beginning of 4 h incubation.
* $p < 0.02$ vs control
** $p < 0.005$ vs LH only
*** $p < 0.001$ vs control

Since gonadotropin-stimulated testicular steroidogenesis depends upon continuous synthesis of new RNA and protein molecules and can be completely inhibited by protein synthesis inhibitors such as cycloheximide (10,11) it was of interest to compare the effect of this inhibitor on ornithine decarboxylase activity and testosterone production. In agreement with previous reports (10,11) cycloheximide dose-dependently and completely inhibited LH-stimulated testosterone synthesis in Leydig cells (Table III). In contrast, LH-stimulated ornithine decarboxylase activity was blocked by the two lowest doses of cycloheximide and further 100-fold increase in the dose of the inhibitor produced no statistically significant additional inhibition of the enzyme activity.

Furthermore, analysis of temporal relationship between LH-stimulated ornithine decarboxylase activity and testosterone production showed that the enzyme activity actually decreased during the first two hours of incubation, compared to freshly isolated cells (4), while testosterone production continued linearly over the entire 4 h of incubation (results not shown).

DISCUSSION

Pituitary gonadotropins, follicle-stimulating hormone and LH, the two chief regulators of testicular function, have been shown to stimulate tes-

TABLE III

Effect of Cycloheximide on Testosterone Production ($ng/10^6$ cells per 4 h) and Ornithine Decarboxylase Activity (pmol/mg protein per 30 min)*

Treatment		Testosterone	Ornithine Decarboxylase Activity
LH (ng/ml)	Cycloheximide (μg/ml)		
0	0	2.22 ± 0.02	32.84 ± 1.54
100	0	11.42 ± 0.22	46.89 ± 1.70[a]
100	0.05	8.78 ± 0.29	36.77 ± 0.51[b]
100	0.25	4.83 ± 0.12	31.85 ± 1.74[b]
100	2.5	2.24 ± 0.04	27.95 ± 1.10[b]
100	25.0	1.64 ± 0.05	26.28 ± 3.20[b]

*Results are means ± S.E.M. of 3 incubations. LH and cycloheximide were added in the beginning of 4 h incubation

[a] $p < 0.02$ vs control

[b] $p < 0.01$ vs LH only

ticular ornithine decarboxylase following in vivo administration to immature rodents (12-14). More recently, we demonstrated for the first time direct in vitro effect of LH on this enzyme in isolated testicular interstitial cells from adult rats (4). The functional significance of enhanced polyamine biosynthesis in the regulation of differentiated testicular functions such as spermatogenesis and steroidogenesis remains poorly understood. The results of the present study suggest that although LH stimulates ornithine decarboxylase and testosterone production in interstitial cells at a similar physiological dose range, there appears to be no apparent functional coupling of these two responses. DFMO, an enzyme-activated irreversible inhibitor of ornithine decarboxylase caused about 59% inhibition of the enzyme activity but produced no statistically significant impairment of testosterone synthesis. The magnitude of the enzyme inhibition by DFMO we observed was similar to that reported by Danzin et al (15) in the rat testis following in vivo administration of this inhibitor. DFMO produced a much higher degree of the enzyme inhibition in porcine ovarian granulosa cells in vitro without affecting LH-stimulated progesterone synthesis (16). The reason(s) for those tissue differences are not well understood. It is of note that though the inhibition of ornithine decarboxylase in the rat testis was of a moderate degree, testicular putrescine level was found to be markedly depleted (15). Whether a similar degree of polyamine depletion occurred in our cell preparation remains to be determined. A recent in vivo study in rabbits in which intratesticular DFMO administration was employed suggested that ornithine decarboxylase activity was not required for testicular androgen production (17).

Cycloheximide in the dose of 25 μg/ml was reported to cause about 95% inhibition of protein synthesis in isolated testis Leydig cell (18). In view of this, it was surprising to find that it did not produce a higher degree of inhibition of ornithine decarboxylase activity. In porcine ovarian granulosa cells in vitro, cycloheximide (10 μg/ml) completely inhibited both LH-stimulated ornithine decarboxylase activity and progesterone production (16). It is possible that in our cell preparation

cycloheximide was affecting both ornithine decarboxylase synthesis and degradation.

In conclusion, in rat testicular interstitial cells there appears to be no causal coupling between LH-stimulated ornithine decarboxylase activity and testosterone production.

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