Results. The serum LH levels in the intact hamsters increased at 15.00 h, fell back to a low level at 16.00 h, then increased sharply at 17.00 h, but were low again at 21.00 h (fig. 1). The GnRH-R was maximal at 13.00 h, and decreased to a low level at 16.00 and 21.00 h (fig. 1).

In the O VX/ADX hamsters, LH levels were maximal at 18.00 h and much lower at the other sampling times (fig. 2). The GnRH-R was generally lower in the O VX/ADX hamsters than in the intact animals, but there was a decrease in the GnRH-R between 12.00 and 16.00 h in the O VX/ADX hamsters similar to the fall in the intact animals (fig. 2).

Discussion. Although none of the animals in the present experiments were maintained on a long photoperiod, the number of GnRH receptors in intact hamsters on LD 6:18 was generally comparable to that reported for normally cycling rats. The cause of the fall in the number of pituitary GnRH receptors preceding the pre-ovulatory gonadotropin surge is perplexing. The decrease is apparently not due to in vivo or in vitro occupation of the receptors by GnRH, and a fall in binding occurs in ovarioctomized rats implanted with estrogen suggesting that changes in serum estrogen levels are not involved. However, there may be time of day changes in estrogen and progesterone levels in ovarioctomized, estrogen implanted animals due to adrenal secretion of progesterone and due to rhythms in the metabolism of estrogens.

The present study shows that there is a fall in the number of GnRH receptors preceding the LH surge in intact or ovarioctomized-adrenalec-tomized female hamsters on short photoperiod. These results indicate that changes in the levels of gonadal steroids are not the cause of the fall in the number of receptors and that elevated estrogen levels are not necessary for the decline to take place.

Key words. Fetus; Leydig cells; androgen; catecholamines; primary culture.

β-Adrenergic stimulation of androgen production by fetal mouse Leydig cells in primary culture

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Summary. The responsiveness of fetal mouse Leydig cells to catecholamines (epinephrine, norepinephrine), a β-agonist agent (L-isoproterenol) and hCG was investigated in vitro. Fetal Leydig cells when freshly isolated were unable to respond to L-isoproteronol, norepinephrine and epinephrine significantly stimulated androgen production by fetal Leydig cells after 24 h of primary culture. Androgen production was increased in both conditions and to a greater extent by hCG.

Material and methods. Fetal Leydig cells were isolated from 18-day-old fetal mouse testes. The complete protocol has been described elsewhere. Briefly, fetal testicular cells were obtained by mechanical dissection and collagenase treatment. Aliquots of the cell suspension were incubated for 2 h in Falcon tissue culture dishes. Medium 199 supplemented with 15 mM Hepes (Eurobio, Paris, France), 0.1% BSA (ICN Pharmaceutical Inc.), glucose, 1% fetal calf serum (Difco Laboratoires, Detroit, USA), 100 U penicillin/ml and 100 μg streptomycin/ml (Difco Laboratoires) was used. At the end of this period, floating cells were removed and firmly attached cells were washed 3 times. About 70% of the cells remained.

References


L-isoproterenol, epinephrine, norepinephrine and DL-propranolol (Sigma Chemical Co, St Louis, USA) were added at the concentrations indicated in the results section. hCG (2nd IS for chorionic gonadotrophin for bioassay, 2180 IU/mg), was used at a concentration which gives maximal testosterone response as previously reported. At the end of the incubation period media were collected, centrifuged for 20 min at 3000 g and frozen at -30°C. Androgens were measured directly in the unextracted media by a testosterone radioimmunoassay as previously described. Results are expressed as means and SE of the means.

Statistical analyses were performed by Student's t-test and Duncan's new multiple range test.

Results. As indicated in figure 1 freshly isolated Leydig cells responded to 100 mIU hCG (panel A) by a 6-fold increase in androgen production (p < 0.001) but were unresponsive to 10^{-5} M L-isoproterenol. In contrast L-isoproterenol stimulation at 24 h of culture (panel B) elicited a significant 2.6-fold increase over basal androgen production (p < 0.001). The total amount of androgen produced by L-isoproterenol stimulation was, however, lower than that produced by stimulation with hCG (p < 0.05). The effects of L-isoproterenol, epinephrine, norepinephrine, and the addition of the β-receptor antagonist, propranolol with L-isoproterenol or epinephrine are shown in figure 2. The original data are presented as percentages of basal androgen production to eliminate differences in basal production between the different experiments. The results indicate that epinephrine and norepinephrine also stimulated androgen production by cultured fetal Leydig cells. At the concentrations used (10^{-5} M), the percentage of stimulation was similar for the three agents tested (p < 0.001). The stimulatory effect of L-isoproterenol or epinephrine on androgen production was totally prevented by concomitant exposure of the cells to the β-receptor antagonist, propranolol. To test the effect of IBMX on androgen production, fetal Leydig cells were incubated, in a subsequent series of experiments, with or without 0.1 mM of this agent. Our results indicate that the levels of androgens produced in the presence or absence of IBMX were not significantly different (control cells: 1270 ± 101 pg/500 µl, n = 4); cells incubated with IBMX: 1225 ± 74 pg/500 µl, M ± SEM, n = 4).

Discussion. The present data indicate that androgen production by isolated fetal mouse Leydig cells can be stimulated by L-isoproterenol, epinephrine and norepinephrine after a 24-h-culture period. Our results confirm and extend the recent report that catecholamines may affect testicular function directly during fetal development by increasing androgen production. Furthermore, our observation that concomitant exposure of fetal Leydig cells to propranolol, a β-adrenergic antagonist, inhibited the androgen response to L-isoproterenol or epinephrine suggests that the action of these neurotransmitters is mediated via β-adrenergic receptors. The reason for the inability of L-isoproterenol, in the present study, to increase androgen production by freshly isolated fetal Leydig cells is unclear. Such a development of L-isoproterenol responsiveness during culture has been previously described for adult gonadal tissue. Although whole decapsulated adult testes or freshly isolated Leydig cells are unable to respond to β-adrenergic stimulation, it was reported that catecholamines stimulate androgen production by adult Leydig cells cultured for 24 h or more.3. Acquisision of catecholamine responsiveness during culture has also been reported for granulosa cells. Although the exact mechanism by which this phenomenon is mediated is not known, it has been hypothesized that the ability to respond to β-adrenergic stimulation during culture is most likely to be the result of an alteration in the state of differentiation, rather than a recovery from a state of β-adrenergic receptor desensitization.

The presence of high levels of epinephrine and norepinephrine in both amniotic fluid and fetal circulation together with our present finding that catecholamines stimulate in vitro androgen production by isolated fetal Leydig cells, suggest that catecholamines may play an essential role(s) in the control of testicular function during fetal development. These studies also demonstrate that the procedures for purification and culture of Leydig cells from fetal mouse testis should be useful for studying further the hormonal regulation of testicular steroidogenesis under controlled in vitro conditions.

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Figure 1. Effect of L-isoproterenol (ISO) and human chorionic gonadotrophin (hCG) on androgen production (M ± SEM) by freshly isolated fetal Leydig cells (panel A) or after a 24-h culture period (panel B). Androgen production was measured in the medium after 3-h incubation in the presence or absence of L-isoproterenol or hCG. The numbers in circles are the numbers of cultures used for each treatment. (***, p < 0.001 vs basal values).

Figure 2. Catecholamine stimulation of androgen production by mouse fetal Leydig cells (M ± SEM). Leydig cells cultured for 24 h, were subsequently incubated for 3 h with 10^{-5} M L-isoproterenol (ISO), 10^{-5} M epinephrine (E) or 10^{-5} M norepinephrine (NE) in the presence or absence of 10^{-3} M propranolol (PRO). Data are presented as percentage of basal androgen production. Broken line indicates basal production. (***, p < 0.001 vs basal values).