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 OVX/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 OVX/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 OVX/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[1,2]. 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[4], and a fall in binding occurs in ovariectomized rats implanted with estrogen[5] suggesting that changes in serum estrogen levels are not involved. However, there may be time of day changes in estrogen and progesterone levels in ovariectomized, 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 ovariectomized-adrenalectomized 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.

1 Savoy-Moore, R.T., Schwartz, N.B., Duncan, J.A., and Marshall, J.C., Science *209* (1980) 942.
2 Clayton, R.N., Solano, A.R., Garcia-Vela, A., Dufau, M.L., and Catt, K.J., Endocrinology *107* (1980) 699.
3 Adams, T.E., and Spies, H.G., Endocrinology *108* (1981) 2245.
4 Savoy-Moore, R.T., Schwartz, N.B., Duncan, J.A., and Marshall, J.C., Endocrinology *109* (1981) 1360.
5 Barkan, A., Regiani, S., Duncan, J., Papavasilou, S., and Marshall, J.C., Endocrinology *112* (1983) 387.
6 Seegal, R.F., and Goldman, B.D., Biol. Reprod. *12* (1975) 223.
7 Bridges, R.S., and Goldman, B.D., Biol. Reprod. *13* (1975) 617.
8 Stetson, M.H., Watson-Whitmyre, M., and Matt, K.S., Biol. Reprod. *19* (1978) 40.
9 Goldman, B.D., Mahesh, V.B., and Porter, J.C., Biol. Reprod. *4* (1979) 113.
10 Bittman, E.L., and Goldman, B.D., J. Endocr. *83* (1979) 113.
11 Pieper, D.R., Endocrinology *115* (1984) 1857.
12 Marshall, J.C., and Odell, W.D., Proc. Soc. exp. Biol. Med. *149* (1975) 351.
13 Clayton, R.N., Shakespear, R.A., Duncan, J.A., and Marshall, J.C., Endocrinology *105* (1979) 1369.
14 Clayton, R.N., and Catt, K.J., Endocrine Rev. *2* (1981) 186.
15 Niswender, G.D., Midgley, A.R. Jr, Monroe, S.E., and Reichert, L.E. Jr, Proc. Soc. exp. Biol. Med. *128* (1969) 807.
16 Bast, J.D., and Greenwald, G.S., Endocrinology *94* (1974) 1295.

0014-4754/86/060615-03$1.50 + 0.20/0

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

G. Pointis and M.T. Latreille

INSERM U. 166, Groupe de Recherches sur l'Endocrinologie de la Reproduction, Maternité Baudelocque, 123, bd de Port-Royal, F-75014 Paris (France), 11 March 1985

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-isoproterenol (10^{-5} M). However, L-isoproterenol, epinephrine and norepinephrine 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. Propranolol blocked the stimulatory effect of L-isoproterenol and epinephrine. It is concluded that catecholamines can regulate fetal testosterone biosynthesis.

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

It is well established that gonadotropins (LH/hCG) are the main hormones which regulate testicular testosterone biosynthesis. In the course of studies of factors influencing the steroidogenic activity of the testis, it was recently reported that catecholamines can exert a stimulatory effect on androgen production by adult interstitial cells in primary culture[2-3], via specific β-adrenergic receptors[4]. The presence of higher concentrations of norepinephrine in the neonatal testis as compared with prepubertal and adult testes[5] has raised the question of whether there is a direct effect of catecholamines on androgen production during fetal development. Recently we have established an in vitro primary culture of fetal mouse Leydig cells that maintains steroidogenic responsiveness to gonadotropin[6]. In this system the direct effect of L-isoproterenol, epinephrine and norepinephrine upon androgen production was examined.

Material and methods. Fetal Leydig cells were isolated from 18-day-old fetal mouse testes. The complete protocol has been described elsewhere[6]. Briefly, fetal testicular cells were obtained by mechanical dissection and collagenase treatment. Aliquots of the cell suspension were incubated for 2 h in Falcon 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 Laboratories) was used. At the end of this period, floating cells were removed and firmly attached cells were washed 3 times. About 70% of the firmly attached cells stained positively for 3β-hydroxysteroid dehydrogenase as described previously[6]. Viability of these cells exceeded 90% estimated by trypan blue staining. Fetal Leydig cells, freshly isolated or cultured for 24 h before any treatment in medium 199 containing 15 mM Hepes, 0.1% glucose/1, 100 U penicillin/ml and 100 µg/ml streptomycin, were then incubated for 3 h at 37 °C in supplemented medium, which contained 0.1 mM 3-isobutyl-1-methylxantine (IBMX), a phosphodiesterase inhibitor which increases endogenous levels of cAMP and 0.1 mM ascorbic acid, to reduce breakdown of catecholamines.

Experientia 42 (1986), Birkhäuser Verlag, CH-4010 Basel/Switzerland

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[7]. 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[6]. Results are expressed as means and SE of the means.

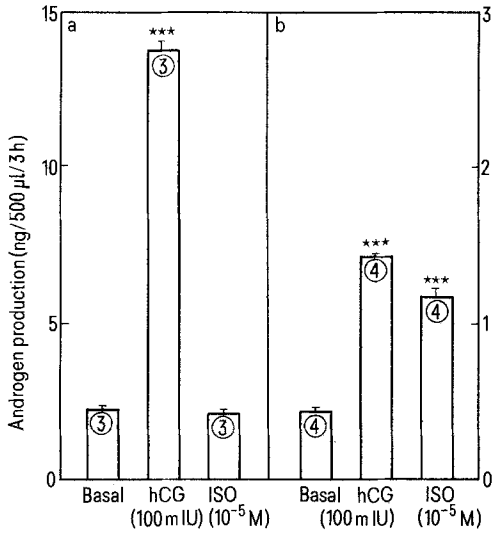

Figure 1. Effect of L-isoproterenol (ISO) and human chorionic gonadotrophin (hCG) on androgen production (M ± SEM) by freshly isolated fetal Leydig cells (panel A) or after a 24-h culture period (panel B). Androgen production was measured in the medium after 3-h incubation in the presence or absence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

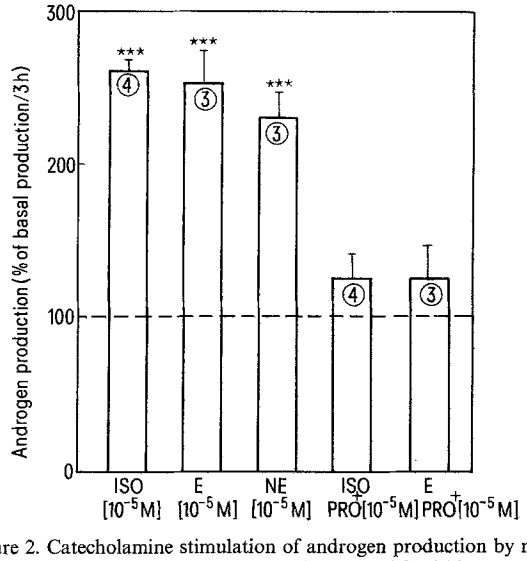

Figure 2. Catecholamine stimulation of androgen production by mouse fetal Leydig cells (M ± SEM). Leydig cells cultured for 24 h, were subsequently incubated for 3 h with 10^{-5} M L-isoproterenol (ISO), 10^{-5} M epinephrine (E) or 10^{-5} M norepinephrine (NE) in the presence or absence of 10^{-5}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

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, epinenephrine 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[8]. 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 neuroactive agents 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[2] or freshly isolated Leydig cells[2-3] 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[2-3]. Acquisition of catecholamine responsiveness during culture has also been reported for granulosa cells[9]. Although the exact mechanism by which this phenomenom 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[10], rather than a recovery from a state of β-adrenergic receptor desensitization[3].

The presence of high levels of epinephrine and norepinephrine in both amniotic fluid and fetal circulation[11-12], together with our present finding that catecholamines stimulate in vitro androgen production by isolated fetal Leydig cells, suggest that catecholamines can 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.

1 We thank the National Institute for Biological Standards and Controls, London, for the hCG standard and M. Verger for help in the preparation of the manuscript.

2 Moger, W.H., Murphy, P.R., and Casper, R.F., J. Androl. *3* (1982) 227.
3 Cooke, B.A., Golding, M., Dix, C.J., and Hunter, M.G., Molec. cell. Endocr. *27* (1982) 221.
4 Poyet, P., and Labrie, F., Biol. Reprod. *28* (1983) 59.
5 Zieher, L.M., Debeljuk, L., Iturriza, F., and Mancini, R.E., Endocrinology *88* (1971) 351.
6 Pointis, G., Rao, B., Latreille, M.T., and Cedard, L., J. Steroid Biochem. *20* (1984) 525.
7 Pointis, G., Latreille, M.T., and Cedard, L., in: Recent Progress in Cellular Endocrinology of the Testis, p. 287. Eds J.M. Saez, M.G. Forest, A. Dazord and J. Bertrand. INSERM, Paris 1985.
8 Anakwe, O.O., and Moger, W.H., Biol. Reprod. *30* (1984) 1142.
9 Kliachko, S., and Zor, V., Molec. cell. Endocr. *23* (1981) 23.
10 Moger, W.H., and Murphy, P.R., Archs Androl. *10* (1983) 135.
11 Zuspan, F.P., Berhman, R., and Paton, J., Am. J. Obstet. Gynec. *118* (1974) 837.
12 Ben-Jonathan, N., and Maxson, R.E., Endocrinology *102* (1978) 649.

0014-4754/86/060617-03$1.50 + 0.20/0

Comparative distribution on niobium-95 in maternal and fetal rats and rabbits[1]

M. Schneidereit and H. Kriegel

Gesellschaft für Strahlen- und Umweltforschung mbH, Abt. Nuklearbiologie, Ingolstädter Landstrasse 1, D-8042 Neuherberg (Federal Republic of Germany), 10 May 1985

Summary. Pregnant rats and rabbits were injected with Nb95 towards the end of gestation. In rats, all maternal tissues showed higher concentrations compared to the fetal organs; the highest ratio was 0.6 in bone. In rabbits a different distribution was found. The fetal bone exhibited a 3.5 times higher concentration of Nb95 than the maternal one.
Key words. Niobium-95 diaplacental transfer; species dependence; bone.

Niobium, a rare earth element, is a trace constituent of stainless steels and an anti-cracking additive in steel compounds of reactor systems. It can be activated to give several isotopes, of which Nb94 has been pointed out to be the most hazardous to the environment because of its half-life of more than 20000 years and its high dose rate[2]. Former work[3] and a previous report[4] on the transfer and distribution of Nb revealed inconsistent results concerning its uptake into the fetal organs of different species. The calculation of the radiological risk to man after incorporation of these nuclides, however, is based on such animal data. The purpose of the work now to be reported was to compare the transplacental movement and the subsequent distribution of niobium in rats and rabbits under the same conditions.

Materials and methods. Virgin female Wistar rats aged 10–12 weeks were caged with males overnight and mating was confirmed by the presence of sperm next morning, at day 0. Groups of 5 animals were injected with 740 kBq Nb95 oxalate (carrier free; $t_{1/2}$ = 35 d) i.v. into the tail vein on day 18 and day 20 of gestation. The animals were killed 24 h later. From each female 5 fetuses were assayed for whole body measurements and 5 were dissected. Blood, liver, kidney and femur of dams and fetuses, and placentas, were removed and weighed. The samples were measured in a well-type scintillation counter and the activity was expressed as a percentage of the injected dose/g of tissue.

Pregnant New Zealand white rabbits at an age of 6 months were injected into the ear vein in groups of 3 animals on days 26 and 28 of gestation, receiving 3.7 MBq Nb95 of the same solution as the rats. They were also killed 24 h after injection and handled as described above. Immediately after injection, rats and rabbits were measured in a whole body counter to confirm the injected dose. The decrease in retention was determined by whole body γ-counting directly before killing the animals 24 h later. All data in this report were corrected for physical decay.

Results and discussion. The tissue concentrations of Nb95 in rats and rabbits are given in the table. Both species were investigated at the same stages of gestation. Calculating an average duration of pregnancy of 22 days for rats and 31 days for rabbits, both species had passed about 82% and 90% of the total gestational period when they were given the injection. For rats this was days 18 and 20, for rabbits it was days 26 and 28 of gestation. Maternal blood and kidneys showed high concentration of Nb in both species, whereas the corresponding fetal tissues demonstrated very low concentrations, leading to low fetal to maternal concentration ratios. Liver concentrations of rats and rabbits were considerably different.

However, the relation between maternal and fetal concentrations was similar in the two species, indicating a comparable biokinetic behavior of Nb in liver. The placentas contained high concentrations of Nb which even increased towards the end of gestation in both species.

The mean whole body concentrations of the mothers were calculated from the Nb retention 24 h after administration of the nuclide. For rats, the retention was 86.6% on day 18 and 84.2% on day 20; the maternal rabbits retained 91.6% and 91.8% of the injected dose on day 26 and day 28 of gestation, respectively. The total body concentration of maternal rats was 5–10 times higher compared with the rat fetuses. In rabbits, the ratio of whole body concentrations was 0.7–0.8, which indicates a similar total body burden of Nb in rabbit dams and fetuses at this gestational stage.

An apparent difference was seen between the femur concentrations of both species. The C_f/C_m in rats was 0.6. In rabbits, however, the ratio was 2.0 on day 26 and 3.5 on day 28 of gestation, thus presenting a different radiological hazard to the fetal bone. Although a former report on the Nb distribution in rabbits[3], quoting a ratio of 100, could not be confirmed, a definite difference between rat and rabbit results became evident. The actual reasons for these divergent results are difficult to find out. With respect to the placenta physiology of both species there were no obvious contradictions. The hemochorial placentas of rats and rabbits are so similar that they are both regarded as suitable models to study diaplacental transfer[5]. Taking into account that direct comparison of rat and rabbit data requests further transformation[6] because of the 10 times higher body mass of rabbits, at least the ratios of fetal to maternal tissue concentrations remain constant so that they can be compared. Bone physiology and histological bone structure in adult rats and rabbits show no particular differences. They both have a low differentiated Haversian system, where secondary Haversian channels are rarely seen[7]. But in the fetal bone development there is a significant difference between these two species. In the rat and the rabbit fetus, ossification of the femur starts at the same time, at about day 18 of gestation[8]. Ten days later, when rabbit fetuses were examined they were in a phase of