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A. Chabab, Ch. Sultan, O. Fenart, J.C. Nicolas and B. Descomps. INSERM U.58 60 Rue de Navacelles 34100 Montpellier - France. AROMATASE ASSAY BY BIOLUMINESCENCE : STIMULATION OF AROMATASE ACTIVITY BY ANDROGENS IN HUMAN SKIN FIBROBLASTS. A sensitive assay of aromatase was developed to study the regulation of this enzyme in cultured human foreskin fibroblasts: estrone and estradiol were determined in the culture medium by bioluminescence (1) before and after incubation of the cells in the presence of 10^{-6} M substrate (Δ_4 androstène-dione In the conditions of the assay the estrogen formation was linear as a function of time and protein concentration. Similar results were obtained by bioluminescence and by the method using "tritiated water" 6.4 ± 0.7 pmol/mg protein/day (pmol/mg/d) versus 7.9 + 1.3 pmol/mg/d (mean ± S.D.). A Km of 92 nM was obtained in cultured fibroblasts derived from genital skin of normal prepubertal boys and aromatase activity did not vary between 1 and 12 serial subcultures. In cultured fibroblasts from normal boys a significant difference ($p < 0.001$) was observed before and after puberty 7.9 ± 1.3 (n = 19) and 24.5 ± 4.5 (n = 11) pmol/mg/d respectively. This pubertal rise in males could suggest an implication of androgens in extragonadal aromatase regulation. We thus studied the aromatase activity in fibroblasts derived from normal pubertal boys after 12, 24 and 48 h stimulation by various concentrations of the non aromatizable androgen 5α dihydrotestosterone (DHT). Aromatase activity was stimulated 3 to 20 fold by a 48 h preincubation with 10^{-10} and 10^{-9} M DHT but no stimulation was detected for shorter incubation times. This stimulation was partially blocked by the anti-androgen cyproterone-acetate and prevented by Cycloheximide or Actinomycine D. These results suggest that DHT increases aromatase activity in cultured fibroblasts through induction of synthesis of new proteic material

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CATECHOLAMINE STIMULATION OF TESTOSTERONE PRODUCTION BY FETAL MOUSE LEYDIG CELLS.

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There is compelling evidence that catecholamines can exert stimulatory effect on Leydig cell function and that this influence in mediated via β-adrenergic receptor on Leydig cells. The high values of norepinephrine detected in the 1-day-old rat testes as compared with prepuberal and adult tissues and the presence of catecholamine in the fetal circulation strongly support the hypothesis that catecholamines may play some important role during fetal and neonatal testicular development. In the present study the effect of β-adrenergic agonist on testosterone production in freshly isolated and cultured Leydig cells from 18-day old mouse fetues was examined. Fetal Leydig cells were obtained by mechanical dissection and collagenase dispersion using the method described earlier (Pointis et al., J. Steroid Biochem., 1984, 20, 525). Testosterone production by fresh fetal Leydig cells was not affected by the presence of isoproterenol, but was significantly stimulated by hCG. Isoproterenol (10^{-5}M), epinephrine (10^{-5}M)and norepinephrine (10^{-5}M) increased testosterone production by fetal Leydig cells after a 24 hrs of primary culture in serum-free medium. A significant effect of isoproterenol on testosterone production could be produced with a concentration as low as 10^{-7}M, which is in the physiological range. The response of the cells to isoproterenol was dose-dependent with a ED_{50} at 2 10^{-7}M. Propranolol, a β-adrenergic antagonist inhibited in a dose dependent manner isoproterenol-stimulated testosterone production, while an α-adrenergic antagonist, phentolamine, had no effect. These results suggest that catecholamines may be involved in the control of testicular steroidogenesis during fetal development.