13

MODULATION OF CULTURED LEYDIG CELL STEROIDOGENIC ACTIVITY BY SERTOLI CELL SECRETED FACTORS.

A.M.MORERA,M.BENAHMED,M.A.CHAUVIN,C.GRENOT and E.de PERETTI.
INSERM U34 and U162 Hopital Debrousse 69322 LYON FRANCE

Using a model of coculture of immature porcine Sertoli and Leydig cells,we have shown that FSH enhances Leydig cell activity via Sertoli cells. Conditioned medium (CM) from FSH-stimulated Sertoli cells (FSH-CM) mimics the effect of FSH-stimulated Sertoli cells,suggesting that Sertoli cells act via diffusible factors. Here, we have investigated the effect of such factors on Leydig cell activity by two parameters: 125-I hCG binding and steroid production (testosterone, DHAS and Pregnenolone sulfate). We have compared the activity of Leydig cells cultured in FSH-CM to CM from control Sertoli cells (C-CM). An increase of 125-I hCG binding and basal and hCG-stimulated steroid production is observed in Leydig cells cultured in FSH-CM versus C-CM. Scatchard analysis of hCG receptors show an increase of hCG binding sites without modification of hCG receptor affinity. Dose-response curve to hCG shows that maximal testosterone response is increased whereas the ED 50 is not modified. The steroidogenic response to hCG,PGE2,8Br cAMP and forskolin is increased. The presence of LDL-cholesterol in the culture medium potentiates the steroidogenic effect of FSH-CM.These results show that FSH-dependent factors from Sertoli cells modulate Leydig cell activity by several mechanisms:1)hCG binding sites number,2)exogenous cholesterol utilisation, 3) response to cAMP stimulation. Precise sites of action of these factors on steroid biosynthesis are under investigation.

14

CONFORMATIONAL CHANGES INDUCED BY STEROID HORMONES BINDING TO SERUM ALBUMIN: A KINETIC STUDY

Gianfranco GIRAUDI - Department of Analytical Chemistry, University of Torino, Torino

The kinetics of the association reaction between bovine serum albumin (BSA) and estradiol (E_2), progesterone (Pg), testosterone (T), 5α-dihydrotestosterone (DHT) and androstenedione (A) was studied at 9.5°C, pH 7.4, 0.15 M ionic strength, by a stopped-flow spectrophotometric technique by following the time course of the fluorescence quenching of the BSA upon steroid binding. For all steroids examined two first-order steps were observed: a fast fluorescence quenching followed by a much slower one. With increasing concentrations of excess steroid, the first-order rate constants showed an enzyme-like saturation kinetics for the first step, but were constant for the second step. This trend provides direct evidence of a complex reaction mechanism of the type: $P + S \overset{1}{\rightleftarrows} C \overset{2}{\rightleftarrows} D \overset{3}{\rightleftarrows} E$, where a very fast formation of an intermediate complex (C) is followed by a minor isomerization step (2) and then by a much slower, more extensive rearrangement (3). The derived equilibrium and kinetic constants (K_1 (l/mol), K_2, K_{eq} (l/mol), k_2 (1/s), and k_{-2} (1/s), respectively) for some steroids are: Pg) $5.8 \cdot 10^4$, 2.6, $2.1 \cdot 10^5$, 8.4, 3.2 E_2) $5.3 \cdot 10^4$, 2.5, $1.9 \cdot 10^5$, 12.5, 5 DHT) $3.6 \cdot 10^4$, 2.0, $1.1 \cdot 10^5$, 20, 10. The first-order rate constants of the slower step (3) are almost independent on steroid structure. The rise of K_{eq} together with the lowering of the forward and reverse rate constants with increasing K_2, shows clearly how a tight binding in the isomerization step can affect both the kinetics and the equilibrium of steroid-protein interactions.