THE EXPRESSION OF PROTEINS INVOLVED IN STEROIDOGENESIS IN THE TESTICULAR CELLS OF RATS OF DIFFERENT AGES

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The goal of this study was to evaluate the expression of StAR protein (Steroidogenic Acute Regulatory protein; one of the most important factors controlling steroidogenesis in the immediate mechanism) and aromatase (the enzyme converting androgens to estrogens, active in Sertoli cells in younger animals, and in Leydig cells from adolescence) in the testicular cells of rats of different ages. In addition, the influence of interleukin-1 (IL-1) and interleukin-6 (IL-6) on the expression of the above-mentioned proteins was studied. Since both interleukins affect apoptosis and necrosis, the experiments were completed with the evaluation of these processes in Leydig cells (LC) subjected to IL-1 and IL-6.

Testicular cells were isolated from 10-, 20-, 30-, 40- and 65-day-old rats by means of digestion with collagenase and percoll gradient centrifugation. LC were incubated with chosen concentrations of IL-1α, IL-1β and IL-6, without or with the presence of LH, for 12h. RNA was isolated from LC and seminiferous tubule cells (STC) using the Chomczynski-Sacchi method. Then, RT-PCR was performed with primers specific for StAR protein and aromatase. The products were analysed by gel electrophoresis. Moreover, the quantification of cDNA synthesis was carried out using fluorescence real-time RT-PCR. To refer the expression of the studied proteins to the synthesis of testosterone, culture media from Leydig cells were collected for testosterone RIA. Some LC subjected to IL-1 and IL-6 were stained with propidium iodate and Hoechst 33342 dye, and the percentage of apoptotic and necrotic cells was counted using a fluorescence microscope.

The results indicate that the expression of StAR protein and aromatase depends on the age of the animals, and exposure to studied cytokines. In addition, they seem to confirm “the switch” of aromatase activity from Sertoli cells to LC in adolescent rats. RIA revealed the inhibition of testosterone synthesis by IL-1 and IL-6 in 40-day-old animals. Younger LC did not respond to stimulation with LH, nor to modulation by interleukins. The data indicate also the role of IL-1 and IL-6 in the regulation of apoptosis and necrosis in LC.

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