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Retinoic Acid Up-regulate the Expression of Steroidogenic Acute Regulatory Protein in K28 Mouse Leydig Tumor Cells

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Retinoic acids (RAs) exert pleiotrophic effects on cellular growth, differentiation, and testicular functions. The primary function of Leydig cells is to produce testosterone whose intratesticular level is critical for spermatogenesis. Steroidogenic Acute Regulatory (StAR) protein is involved in transporting cholesterol from outer mitochondrial membrane to matrix side of inner mitochondrial membrane where cholesterol side chain cleavage (SCC) enzyme reside. Thus, the StAR protein is essential for acute response of steroidogensis in steroidogenic tissues and the action of the protein is thought to be rate-limiting step for the testosterone synthesis. In order to understand how RAs control steroidogenesis in Leydig dells, cultured mouse Leydig tumor cells K-28 were treated with RAsm and the mRNA levels of StAR gene were monitored by Northern blot analysis. Our study showed that RAs up-regulate the StAR mRNA in K-28 cells in time- and dose-dependent manner while the levels of the SCC mRNA were unchanged. The amount of progesterone produced from RA treated K-28 cells was increased concomitantly with the level of StAR mRNA, which indicates that StAR protein is the key regulator for steroidogenesis.
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Molecular Cloning of Rat Steroidogenic Acute Regulatory Protein and Its Constitutive Expression in Rat Leydig Tumor Cells, R2C

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The expression of steriodogenic acute regulatory (StAR) protein is up-regulated in steroidogenic cells in acute response to trophic hormones and the protein is responsible for the mobilization of cholesterol from mitochondrial outer membrane to inner membrane where cholesterol side chain cleavage enzyme reside. Rat complementary DNA corresponding the StAR protein was cloned and the complete nucleotide sequence was determined. The cDNA is consisted of 1560 nucleotides and has the longest open reading frame of 320 amino acids. The deduced amino acids sequence show high degree of homology to mouse, bovine, and human StAR protein in amino acid level. Rat tissue Northern blot showed that it is only expressed in testis, ovary, and adrenal gland. Rat Leydig tumor cells, R2C can produce testosterone without trophic hormone stimulation or cAMP analog treatment. In order to correlate the constitutive production of the hormone with the the level of StAR mRNA, we analyzed the expression of the StAR mRNA in rat R2C cells. The Northern blot showed that R2C cells expressed the StAR mRNA without stimulation and treatment of cAMP did not increase the level of StAR mRNA, which indicates that constitutive steroidogenisis are caused by high basal level expression of StAR mRNA and StAR expression in R2C cells is not dependent on cAMP level.
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