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THE PATHOPHYSIOLOGY AND ACTION OF THE STEROIDOGENIC ACUTE REGULATORY PROTEIN, STAR

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Steroid hormones are required for homeostasis and reproduction. Steroidogenesis is regulated by two distinct mechanisms. Chronic regulation, determining net steroidogenic capacity, is via transcription of the gene for P450scc, the first and rate-limiting steroidogenic enzyme. Acute regulation, determining responses to stress, is via the steroidogenic acute regulatory protein (StAR), which regulates the flow of cholesterol into mitochondria where P450scc converts it to pregnenolone. Mutation of StAR causes congenital lipoid adrenal hyperplasia (Science 267:1828,'95), the most severe defect in human steroidogenesis. Affected 46,XY genetic males have a female phenotype due to absent fetal testicular steroidogenesis, and individuals of both sexes experience potentially lethal neonatal glucocorticoid and mineralocorticoid deficiency, but survive to adulthood with proper replacement therapy (Clin Endo 23:481,'85). The pathophysiology of lipoid CAH is explained by the two-hit model (NEJM 335:1870,'96). In the absence of StAR (first hit), most steroidogenesis is lost, but low levels of StAR-independent steroidogenesis persist. The consequent increases in ACTH and LH hyperstimulate the adrenal and gonad, causing accumulation of intracellular cholesterol and consequent cell death (second hit). Affected 46,XX females feminize normally and have menarche as the fetal ovary makes no steroids (JCEM 63:1145,'86), hence the ovary is spared from the second hit until adolescence (JCEM 82:1511,'97). StAR is synthesized as a 37kDa cytoplasmic phosphoprotein that is cleaved to 30kDa upon import into mitochondria. Experiments deleting the mitochondrial import signal (PNAS 93:13731,'96) and experiments immobilizing StAR on the outer mitochondrial membrane (OMM), the intramembranous space, or the mitochondrial inner membrane, have now proven that StAR acts exclusively on the OMM, and that its level of activity is proportional to the time it resides on the OMM and inversely proportional to the speed of its mitochondrial importation (Nature 417:87,'02). *In vitro,* StAR undergoes a pH-dependent structural transition to a molten globule at ~pH4, both in aqueous solution (PNAS 96:7250,'96) and in association with synthetic membranes (JBC 276:17044,'01), suggesting that StAR undergoes a conformational change when interacting with acidic head groups of OMM phospholipids, exposing the hydrophobic cholesterol-binding pocket of StAR. Liposome protection experiments show that only the C-terminal a4 helix associates with membranes (JBC in press). Although the precise mechanism of StAR's action remains unknown, StAR is of central interest, as it is the first and best-studied member of a larger family of proteins containing StAR-related lipid transfer (START) domains. These START domain proteins participate in many cellular processes.

THE NEUROBIOLOGY OF FEMALE PUBERTY

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Mammalian puberty is initiated by an increase in luteinizing hormone-releasing hormone (LHRH) secretion from the hypothalamus. This increase is caused by changes in the activity of neuronal and astroglial networks connected to LHRH neurons. The main neuronal systems involved in the facilitation of LHRH secretion at puberty

are those that use excitatory amino acids and the peptide kisspetin/metastin for neurotransmission/ neuromodulation. The main inhibitory neurotransmitter controlling LHRH release is GABA, which exerts these modulatory effects via $GABA_A$ receptors. Astrocytes regulate LHRH secretion through paracrine-autocrine signaling pathways initiated by TGFá and neuregulins (NRGs), two members of the epidermal growth factor family, acting via the receptor tyrosine kinases erbB-1 and erbB-4, respectively. In addition, activation of ependymoglial (tanycytic) erbB1 receptors is an important determinant of the tanycytic plasticity required for LHRH axons to release the decapeptide into the portal system at the time of the preovulatory LHRH surge. Glutamatergic neurons coordinate glial and neuronal activity by facilitating astrocytic erbB-dependent signaling via glutamate AMPA and metabotropic receptors expressed on astroglial cells. Genetic disruption of erbB-1 or astrocytic erbB-4 signaling in mice delays female sexual development due to impaired erbB ligand-induced glial PGE_2 release. The combined defect markedly exacerbates the delay in puberty, indicating that erbB-1 and erbB-4 receptors act coordinately to facilitate LHRH secretion during female sexual development. Quantitative proteomics analysis of the hypothalamus of mice with delayed puberty due to defective astrocytic erbB-4 receptors and global gene expression profiling of the primate hypothalamus during the onset of female puberty demonstrated increased pubertal expression of a family of genes previously known as having «tumor suppressor activity». Important components of this network are the cell-cell adhesive molecule SynCAM, the ligand-receptor complex KiSS1/GPR54, and the transcriptional regulator TTF-1.The developing monkey hypothalamus also expresses three large families of genes encoding proteins involved in synaptic specification, synaptic connectivity, and glial-neuronal communication. Thus, the initiation of female puberty appears to require changes in neuron-glia communication involving excitatory amino acids and glial growth factors, potential changes in synaptic make-up, and an upstream level of control that brings about changes in the transcriptional activity of subordinated genes involved in neuron-to-neuron and neuron-glia bidirectional communication. (Supported by NIH grants HD-25123, RR00163 and NICHD-U54-18185).

CRECIMIENTO Y FUNCION DEL TESTICULO HUMANO PREPUBERAL

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Para este estudio se dividió la prepubertad en las siguientes etapas: 1) una **etapa neonatal**, (Gr1), durante el primer mes de la vida, en la que el testículo está aún influenciado por las hormonas del embarazo y los cambios que suceden durante el parto, 2) una etapa de **activación post-natal** del lactante temprano, o minipubertad, (Gr2), que se extiende hasta los 6-8 meses de edad, y 3) una etapa de **niñez** o cuasi quiescencia prepuberal**,** (Gr3), desde 8 meses de edad hasta la prepubertad tardía, a una edad variable, generalmente entre 11 y 13 años. Este larga tercera etapa puede subdividirse en niñez temprana (hasta los 5 años de edad) y tardía (luego de los 5 años de edad), en base a un moderado aumento del volumen testicular.

Se estudió la apoptosis (AI) por el método de TUNEL y la inmunoexpresión de la proteína Ki-67 (CPI), un marcador del ciclo celular, el IGF-1, receptor IGF tipo 1 (IGFR), la aromatasa (ARO) y los receptores de estrógenos (ERá y ERâ), en las células interticiales (IC), de Sertoli (SC) y germinales (GC) de los tres Gr. En las IC, SC y GC el AI del Gr1 fue significativamente más bajo que en el Gr2 y en el Gr3. Por otro lado, el CPI en las GC fue significativamente más alto en el Gr1 que en los otros Gr. Además, los testículos fueron pesados, como un indicador del volumen testicular en los tres grupos. Se constató que el peso aumentó en el período neonatal, y luego se mantuvo estable.

La expresión del IGF-1 en los tres tipos celulares de los tres Gr fue baja, e indetectable en las SC, que son mayoritarias en los cordones seminiferos, lo que sugiere un escaso papel del IGF-1 local en el crecimiento del testículo en esta edad. La expresión del IGFR mostró las siguientes diferencias: en las IC del Gr1 (12.5±0.42 %) fue significativamente mayor que en el Gr2 (4.79±0.1 %) y o en la edad niñez temprana (1.14±0.45 %). El IGFR no se expresa en las SC, pero si en las GC (10-12 %), sin que haya diferencias entre los grupos de edad. El nivel bajo de IGF-1 local sugiere un papel para los IGFs periféricos en las IC, en el período neonatal..

En el Gr 1 y en el Gr2, la ARO se expresó fuertemente en las IC (39-40 %) y en las GC (31-27 %) pero escasamente en las SC (4-8 %). En la rata inmadura, en cambio, la actividad de ARO se localiza en las SC, pera variar a las IC en la madurez. En el Gr 3 la expresión en IC y GC descendió fuertemente. La expresión del ERá fue siempre baja, en cambio la del ERâ alta en todas las

células del Gr1 (IC 26, SC 24, GC 45 %), Gr2 (IC 12, SC 26, GC 42 %) y Gr3 (IC 14, SC 26, GC 57 %).

En conclusión, el testículo neonatal crece fuertemente, y lo hace por inhibición de la apoptosis. Este crecimiento sería independiente de gonadotropinas, pero estaría modulado por los sistemas IGF/IGFR y estradiol/ERâ, entre otros. Los estrógenos sintetizados en las IC y en las GC a partir de la testosterona, podrían difundir a todas las células testiculares. Finalmente, los estrógenos generados en las GC podrían participar del freno en la espermatogénesis, en la etapa pre-meiotica, propio del testículo fetal y prepuberal.

DIFFERENTIAL ESTROGEN RECEPTOR ACTION IN ENDOCRINE PHYSIOLOGY

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Estrogen receptors (ER) are thought to play a crucial role in development, reproduction and normal physiology. Use of gene targeting, has allowed us to produce lines of mice homozygous for the disrupted ERa gene (aERKO) and ERb genes (bERKO). RPA analysis detected comparable levels of ER-b?mRNA in tissues of aERKO mice suggesting that ER-b expression is not dependent on ERa. aERKO mice were totally unresponsive to uterotropic assays with estradiol, hydroxy TAM, DES. Further support came from the failure of estrogen, EGF or IGF-1 treatment to induce DNA synthesis in aERKO uteri, even though EGF and IGF-1 signaling was shown to be intact by stimulation of c-fos, or IRS-1 protein phosphorylation. Progesterone receptor mRNA was detected in aERKO mice, but not stimulated by estrogen in the uterus, mammary gland and ovary, indicating an estrogen dependent and independent gene regulation. aERKO females are infertile and have hypoplastic uteri and hyperemic ovaries with no apparent corpora lutea. The aERKO ovarian phenotype occurs developmentally and can be reversed by a GnRH antagonist. Ovarian gonadotropin receptor levels, serum estrogen, androgen and LH levels are elevated compared to WT and bERKO females. aERKO but not bERKO females have a loss in estrogen protection from ischemia and atherosclerosis. Findings in bERKO females assessed by continuous breeding studies and superovulation show arrested folliculogenesis and subfertility. Stimulation using *in vitro* follicle cultures indicates that aERKO follicles respond comparable to WT but that bERKO follicles are poor ovulators. Ovarian tissue analyses indicate differential gene expression related to ovulatory stimulation. Analysis of the mammary glands of adult aERKO females showed a primitive ductal rudiment rather than the fully developed ductal tree seen in WT or bERKO mice. aERKO males are also infertile, with atrophy of the testes and seminiferous tubule dysmorphogenesis resulting in decreased spermatogenesis and inactive sperm. Sperm transplantation studies of aERKO males rescues the infertility phenotype. Males have reduced bone density and some alterations in cardiovascular function. Phenotypic differences were seen in sex and aggressive behavior in both aERKO males and females compared to the patterns in WT or bERKO mice. In contrast to the aERKO, the bERKO males are fertile with normal sexual behavior. Recent development of a viable double ER a/b? knock out shows a unique ovarian phenotype of transdifferentiation of granulosa to sertoli cells, suggesting that both ER signal mechanisms must be functional in order to maintain the proper differentiation state of the granulosa cells. Further characterization of the mice and comparison of the individual and double ER gene KO phenotypes will be required to more fully understand the physiological consequences of ER mediated actions and the specific roles of the two different forms of ER in estrogen hormone responsiveness.