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PRE NATAL TESTOSTERONE LEVELS IN XXY AND XYY MALES.

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It has been postulated that behavioural differences between normal males and those with an additional X or Y chromosome may be related to hormonal variations pre or post natally. We have previously demonstrated that XXY males have a testosterone surge in the neonatal period (1) and that during childhood salivary testosterone levels are slightly higher than in control males (2).

The pre-natal hormone status is now investigated using amniotic fluid obtained at ante natal diagnosis between 16 and 20 weeks gestation age in a collaborative project between the Departments of Medical Genetics of Guy's Hospital, London, Oxford, Glasgow and Edinburgh.

The (geometric) mean testosterone levels and ranges (p mol/L) were XY 433.5, range 165-1027 (n=25); XYY 500.0, range 224-1092 (n=15); XXY 419.8 range 87-1021 (n=17); XX 146.9, range 41-474 (n=13).

After log transformation, using the t test no significant differences were found between XY, XYY and XXY males but females had lower testosterone levels ($p<0.001$). These findings give no support to the hypothesis that pre-natal testosterone levels contribute to later behavioural characteristics.

1 Ratcliffe SG. Proc Roy Soc Med, 1976, 69, 189-191.
2 Ratcliffe SG, Butler GE, Jones M. BDOAS, 26, 1-44.

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EXCLUSION OF THE GHRH GENE AS A CANDIDATE FOR MOST CASES OF FAMILIAL ISOLATED GROWTH HORMONE DEFICIENCY

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Familial isolated growth hormone deficiency (IGHD) can have autosomal recessive (type I), autosomal dominant (type II) or X-linked (type III) modes of inheritance. While some cases of IGHD are due to deletions or point mutations affecting the growth hormone (GH-1) gene, linkage studies have excluded the GH-1 gene as the locus responsible for a significant proportion of IGHD cases. This fact, along with the positive response in GH secretion after exogenous GH releasing-hormone (GHRH) administration in several affected subjects, suggests that the GHRH gene is a good candidate for some autosomal cases of IGHD. In order to determine the proportion of IGHD cases that may be due to GHRH gene mutations, we studied three highly polymorphic microsatellites (CA repeats) previously mapped close to GHRH on chromosome 20 by linkage. Using these microsatellites as markers for the GHRH locus, we have carried out linkage analysis in 20 unrelated IGHD families (9 type I, and 11 type II). All available family members were genotyped for the marker D20S44 (no recombination with GHRH at a LOD score of 3.6). Noninformative families were also genotyped for other microsatellite polymorphisms, D20S45 and D20S54 (located at 1 and 2 cM from GHRH, respectively). The segregation pattern of alleles at each of the three loci was compared with that of the disease phenotype. We found at least one recombinant meiosis with discordance between phenotype and genotype in 19/20 (95%) of the families; one family with IGHD II was not informative with any of the markers. Our results were consistent with exclusion of linkage between the GHRH and IGHD loci in all 19 families that were informative. There is a minimal probability (1-2%) of a false negative result due to recombination between the marker and the GHRH gene in each of the 7 families that were not informative with D20S44. Our data indicate that most mutations responsible for IGHD are not within or near the structural gene for GHRH on chromosome 20. Since linkage to GH-1 had also been previously excluded in 40% of these families, mutations in other loci different from GH and GHRH must be the cause of IGHD in these patients.

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ESTROGEN EFFECTS IN NEURAL CELLS *IN VITRO*: MORPHOLOGIC CHARACTERIZATION OF ESTROGEN RECEPTOR (ER)-TRANSFECTED PC12 CELLS. R.H. Lustig and P.W. Baas, Depts. of Pediatrics and Anatomy, University of Wisconsin, Madison, WI 53792.

Estradiol (E_2) induces neurite outgrowth, neuritic spine development and synaptogenesis in ER-containing areas of the rat brain. Such changes may modulate E_2 and gender effects on cognition, behavior, and reproductive physiology. Unfortunately, most studies to date have been performed *in vivo*, and hence elucidation of the cellular effects of E_2 on neurons has been difficult. For this reason, we have developed an *in vitro* model using PC12 rat pheochromocytoma cells, which become postmitotic and and extend neurites in response to Nerve Growth Factor (NGF). Wild-type PC12 (CTL1) cells were stably transfected with the vector pCMV-ERα-neo (JNCI 84:580, 1992) in an attempt to overexpress ER (SER8); or with the control vector pCMV-neo (NEO9). Exposure of NEO9 cells to 1-100 ng/ml NGF caused them to extend neurites in a dose-dependent fashion similar to CTL1 cells, but without neuritic spines or intercellular connections. Coadministration of 10^{-10}—10^{-9} M E_2 to NEO9 cells yielded no additional effects on neurite frequency, nor induction of neuritic spines nor intercellular connectivity. Exposure of CTL1 cells to NGF led to similar increases in neurite frequency, but coadministration of E_2 led to additive increases in neurite frequency, and also to dose-dependent increases in neuritic spine frequency and intercellular connections. SER8 cells demonstrated similar but augmented effects and at lower doses of E_2. Preliminary EM studies of intercellular connections in CTL1 and SER8 cells demonstrate synaptic clefts, post-synaptic densities, synaptic vesicles, and polyribosomal clusters suggestive of synaptogenesis.

These results support the *in vivo* observations of estrogen effects on neurite outgrowth, spine development, and synaptogenesis, and extend them *in vitro*. Thus, estrogen appears to activate an inherent neural morphologic program which affects cellular differentiation. Moreover, these cell lines form the basis of an *in vitro* model for elucidating the mechanisms by which estrogen induces its effects on neuronal differentiation, and will be utilized in future studies of sex hormones and neura organization and activation.

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INDUCTION OF GALANIN GENE EXPRESSION IN GnRH NEURONS WITH PUBERTY IN THE FEMALE RAT. D Marks, A Bagcac, D Clifton, R Steiner. Ob/Gyn & Physiol/Biophys, U.Wash, Seattle WA 98125, USA

Puberty is characterized by increasing plasma levels of gonadotropins, which are thought to be mediated by an increase in the pulsatile secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus. Although hypothalamic content of GnRH increases with puberty, cellular levels of its precursor mRNA remain stable indicating that overall biosynthetic demand for GnRH does not change as a function of puberty. While alterations in afferent activity at the GnRH neuron have been suggested as a mechanism for modulating the release of GnRH at puberty, changes intrinsic to the GnRH neuron itself also remain as plausible governing events. Galanin is a recently described gut-brain peptide that is colocalized with GnRH in hypothalamic neurons where it is thought to play a role in the orchestration of gonadotropin release. We tested the hypothesis that the expression of the galanin gene in GnRH neurons is altered during puberty in female rats. To accomplish this, we sacrificed groups of prepubertal (25 day; n=5) and adult (70 day; n=6) female rats and utilized double *in situ* hybridization and image analysis to estimate and compare between groups the number of GnRH mRNA expressing neurons that coexpress galanin mRNA. While the number of GnRH neurons identified was not different between groups (prepubertal 27 ± 4 vs. adult 30 ± 3) the number of GnRH neurons coexpressing galanin mRNA was greatly increased after puberty (prepubertal 6 ± 3% vs. adult 45 ± 9%; $p<0.006$). **Conclusion**: The apparent increase in galanin biosynthesis in GnRH neurons may subserve the augmentation of pulsatile GnRH release driving the onset of puberty in the rat.

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GROWTH HORMONE (GH) RECEPTOR GENE EXPRESSION IN THE HYPOTHALAMUS IS INCREASED DURING PUBERTY. KA Burton, EB Kabigting, RA Steiner, DK Clifton. Depts of Ob/Gyn & Physiol/Biophys, U. Wash, Seattle WA 98195, USA.

The development of a pulsatile pattern of GH secretion occurs at puberty. Although the mechanisms responsible for the generation of GH pulses are unknown, a direct feedback effect of GH on the hypothalamus (via GH receptors) appears to play an important role. We tested the hypothesis that GH receptor gene expression changes over puberty by comparing GH receptor mRNA levels in the periventricular nucleus (PeN) of prepubertal (25 days) and adult (70 days) male rats. We performed *in situ* hybridization on coronal brain slices using a ^{35}S-UTP-labeled cRNA probe and measured autoradiographic silver grains above GH receptor mRNA-containing cells. We observed that cellular levels of GH receptor mRNA (reported as grains/cell±SE) were significantly higher in adult compared to prepubertal animals (116±6 vs 92±4, respectively; $p<0.05$). The factors responsible for this pubertal increase in GH receptor message are unclear; however, mean plasma levels of GH increase during puberty and GH has been shown to increase GH receptor mRNA levels in other tissues. Therefore, we tested the hypothesis that GH regulates GH receptor gene expression in the PeN by comparing cellular levels of GH receptor mRNA among groups of control and GH-deficient rats (both dwarfs and GHRH antibody-treated). GH deficiency did not significantly alter the GH receptor mRNA levels when compared with control levels. **Conclusion:** An increase in GH receptor gene expression may be involved in the pubertal development of pulsatile GH secretion. This increase in GH receptor mRNA most likely occurs independent of increased mean plasma GH levels.

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MAMMOSOMATOTROPH ADENOMA AND GIGANTISM IN AN 8-Y OLD BOY: PATHOPHYSIOLOGICAL ASPECTS. J-M. Dubuis, C. Deal, C. Goodyer*, R. Drews, S. Asa**, G. Van Vliet, and R. Collu, University of Montreal and McGill University*, Montreal, and University of Toronto**, Toronto, CANADA.

We studied an 8-year old prepubertal boy with growth acceleration (without bone age advancement) from age 3 y. Past history was remarkable for onset of strabismus (age 2 y) and progressive loss of vision, coarsening of features, and no galactorrhea. CT-scan and MRI showed a large intra- and suprasellar mass. Elevated basal GH (x = 28 µg/L) and PRL (x = 120 µg/L) were observed, as well as paradoxical responses of GH to L-Dopa, TRH and OGTT. PRL was reduced by L-Dopa but not modified by TRH. GHRH stimulated release of GH, as well as PRL to a lesser degree. Transsphenoidal partial tumor resection was performed, and subsequent treatment with bromocriptine decreased GH and PRL levels. Complete tumor removal was later achieved by a transcranial approach. Immunohistochemistry and electron microscopy using the immunogold technique confirmed the diagnosis of mammosomatotroph adenoma. Occasional tumor cells were also positive for the α-subunit of glycoprotein hormones and β-TSH. Immunoblots of G-protein subunits in tumoral tissue showed increased α_{s}-42 and α_{s}-47, decreased α_{i}-3 and α_{q}, and no change in α_{o} levels when compared to normal human pituitary tissue. Adenylate cyclase activity was markedly elevated in tumoral tissue and could be further stimulated. Using the polymerase chain reaction and single-strand conformational polymorphism analysis, no mutations in the Gsα gene were found. Tumor explants continued to secrete high levels of GH and PRL, as well as α-subunit of glycoprotein hormones and β-TSH, for up to 22 days in culture. The stimulatory factors (GHRH, TRH) induced dose-related responses. In contrast, the inhibitory factors (SRIF, L-Dopa) were effective in suppressing either basal or stimulated hormone release only at high (µM) concentrations, possibly due to the G-protein anomalies. These results are compatible with an oncogenic process induced by excessive GHRH stimulation of adenylate cyclase-regulated cell hyperactivity, although constitutive activation of specific regulatory factors such as Pit-1 may also play an important role.