15 PREMALIGNANT HISTOLOGICAL CHANGES OF THE GONADS IN INTERSEX CHILDREN WITH A Y-CHROMOSOME. Jørn Müller, Niels E. Skakkebaek. Rigshospitalet, Lab of Reproductive Biology Y, Dept of Pediatrics C, and Hvidovre Hospital, Dept of Pediatrics, Copenhagen, Denmark.

Intersex children with a Y-chromosome carry an increased risk of developing germ cell tumors in adulthood. Carcinoma-in-situ (CIS) of the testis and gonadoblastomas have previously been shown to precede germ cell cancer; therefore, we have investigated gonadal specimens from 17 prepubertal and pubertal individuals without any sign of tumor (12 with the androgen insensitivity syndrome (AIS), 4 with 45,X/46,XY gonadal dysgenesis (GD), and 1 with 46,XY GD), in order to establish whether such histologic changes could be detected at this age. 3 out of 8 consecutive patients with incomplete AIS, aged 2 mo, 13 yrs, and 14 yrs, respectively, showed typical CIS changes, whereas non of those with complete AIS were found to have CIS. All 4 patients with 45,X/46,XY GD (3 with male phenotype, 1 with ambiguous genitalia age 1 mo-17 yrs) had CIS in at least 1 gonad. In all instances of CIS the premalignant nature of the lesion was confirmed by DNA analysis showing aneuploidy of the CIS germ cell nuclei. The 13-yr-old individual with 46,XY GD was a phenotypically normal female who during surgery for appendicitis was shown to have a dysplastic uterus, bilateral Fallopian tubes, and bilateral gonadoblastomas. In the light of evidence that CIS and gonadoblastomas are premalignant conditions, we suggest that all intersex patients with a Y-chromosome should have a gonadal biopsy performed; the information obtained will assist in the decision regarding need for gonadectomy.

16 INCOMPLETE MALE DIFFERENTIATION: PREDICTING THE RESPONSE TO ANDROGEN THERAPY. Trevor A. Hughes and Bronwen A.J. Evans. University of Wales College of Medicine, Dept. of Child Health, Cardiff, UK.

The concentration (Bmax) and binding affinity (Kd) of the androgen receptor (AR) in normal genital skin fibroblasts (GSF) is 775 ± 185 x 10^{-15}moles/ug DNA (mean ± SD, n 22) and 0.88 ± 0.35 x 10^{-10}M respectively. Normal GSF when preincubated with 10nM [^{3}H]-DHT for 20 hr before assay show a mean 2.4-fold (range 1.5 - 5.0) augmentation in AR binding. The in vitro response was tested in GSF obtained from patients with:- complete androgen insensitivity syndrome (CAIS, n 2); partial AIS (PAIS, n 8); isolated hypospadias (IH, n 9); isolated micropenis (IM, stretched penile length <2.5 SD, n 8). Those with CAIS/PAIS who were AR negative, deficient or positive (but qualitatively abnormal) did not augment; 2 PAIS patients given androgens did not virilize. Another 4 patients with PAIS and a normal AR which augmented, did virilize with androgen therapy. All patients with IH and IM had a normal Bmax and Kd broadly except one severe IH with a reduced Kd (2.9 x 10^{-10}M). Each cell line augmented normally (range 1.5 - 2.6). Several IM patients are currently receiving androgens. Preliminary results show that this in vitro bioassay may predict androgen responsiveness in vivo soon after birth in infants with severe incomplete masculinization. The test may also indicate whether some patients with mixed IH/IM may benefit from androgen therapy to promote phallic growth prior to plastic surgery.

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SPECIFIC BINDING OF [^{3}H]-17β ESTRADIOL ([^{3}H]-E_2) TO RABBIT CARTILAGE AT PUBERTY.

Estrogens play an important role in skeletal growth maturation, but a direct effect of these hormones or the presence of estrogen receptors in cartilage at puberty was not well documented. In the present work, E_2 binding was studied in cartilage from male and female rabbit at puberty. Cartilage tissue or cultured chondrocytes were homogenized in 0.6M KCl buffer. Soluble proteins were labeled with [^{3}H]-E_2 and bound and free steroids were separated using hydroxylapatite batch procedure. Scatchard analysis showed a single class of high affinity binding sites with a K_D at 0.1 nM and a number of binding sites 100 to 1000 fmoles/mg DNA in both sexes. The specific binding was temperature and time dependent. E_2, DES, IAM competed with [^{3}H]-E_2 for binding sites, but not progesterone, dexamethasone nor 5α-androstanediol. A slight interaction of R1881 with [^{3}H]-E_2 binding sites was observed. It can be suggested that rabbit cartilage at puberty is a target tissue for estrogens with an affinity constant of E_2 for its binding sites similar to those found in other target tissues. No difference between male and female specific binding was observed.

18 NEITHER TESTOSTERONE NOR FSH ARE RESPONSIBLE FOR DECREASED PRODUCTION OF AMH BY BOVINE SERTOLI CELLS IN PRIMARY CULTURE. Bernard Vigier, Jean-Yves Picard, Jacqueline Campargue, Nathalie Josso, INSERM, Unité de Recherches de Génétique Mécidale, Paris, France.

Production of anti-Müllerian hormone (AMH) by Sertoli cells is maximal during the fetal and neonatal period, and tapers off in the course of postnatal development. To determine the factors responsible for the repression of AMH secretion after birth, Sertoli cells, isolated from immature calves, were plated in an hormonally defined medium and cultured in the presence of 0.1 mM MIX. AMH was assayed in the culture medium using a competition-type RIA capable of detecting 1.5 ng. The proportion of neosynthesized AMH was calculated by determining the amount of immunoreactive AMH unaffected by cycloheximide treatment in conditions where 92 % of protein neosynthesis is inhibited. AMH production by Sertoli cells, expressed per 24 hrs and per 10^6 cells, fell from 90 ng on the day following plating to 1.5 at day 7. The proportion of AMH synthesized after day 1 was 35 % on day 2 and 88 % on day 3. FSH, 2.5 μg/ml, and testosterone, 2 μM, were added to the culture medium on days 2 and 3, immunoreactive AMH was measured in the culture medium at day 3. Mean daily production of AMH by 10^6 Sertoli cells was 28.6±2.6 ng in control cultures, 24.6±2.8 in FSH-treated ones and 26.6±3.4 after testosterone treatment. Differences were not significant by analysis of variance. In the same conditions, FSH increased cyclic AMP production approximately tenfold. Further studies are in progress to determine which factors are essential to the continued production of AMH by Sertoli cells in vitro.

19 DEVELOPMENTAL PATTERN OF ANDROGEN METABOLIZING-ENZYMES AND ANDROGEN BINDING CAPACITY IN HUMAN FORESKIN FROM BIRTH TO PUBERTY. Gérald Theintz, Thierry Steimer & Pierre C. Sizonenko. Div. of Biology of Growth & Reproduction, University School of Medicine, Geneva, Switzerland.

Tissue sensitivity to circulating androgens changes during development. A study of enzymes and androgen receptors in human target tissue from birth to puberty may provide useful information concerning the relative importance of the molecular mechanisms involved. The activities of 17β-hydroxysteroid dehydrogenase [17HSD], 5α-reductase [R], 3α- and 3β-hydroxysteroid dehydrogenases [3HSD's], as well as specific binding to androgen receptors were measured in the 800g supernatant of 49 foreskin homogenates from 8 age groups: pool I, newborns; II, 6-24w; III, 6-12m; IV, 1-3y; V, 3-8y; VI, 8-11y; VII, 11-14y; VIII, > 14y. The activity of 17HSD is very low at birth and then increases to reach the highest level in the older age group, whereas R shows 2 peaks of activity, one maximal at birth and another in the 8-11y age group. The activity of 3HSD's appears to follow the same pattern as 17HSD. In contrast with these marked enzymatic changes, little variation is observed in the binding capacity of non nuclear androgen receptors. In conclusion, our data suggest that, during development, the regulation of androgen action in human foreskin may be more dependent on changing ratios of active [e.g. dihydrotestosterone] versus inactive [e.g. 5α-androstanedione] metabolites than on a modification of receptor binding capacity.

20 EFFECT OF CYPROTERONE ACETATE ON AROMATASE ACTIVITY IN CULTURED FORESKIN FIBROBLASTS: PREVENTION OF INDUCTION BY DEXAMETHASONE

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Aromatase activity of human foreskin fibroblasts (FF) can be determined by analysis of $(^3H)H_2O$ released upon conversion of (1-^{3}H) androstenedione (A) to estrone. FF were incubated with MEM containing (1-^{3}H)A (150nM) and progesterone (5μM) for 8h. Preincubation (37C, 12h) of cells with 250nM dexamethasone (Dex) in medium with FBS or $(Bu)_2$c-AMP (1mM) in medium without FBS resulted in a increase in aromatase activity. When cells were preincubated with Dex and cyproterone acetate (CA), testosterone (T), dihydrotestosterone (DHT), or Estradiol (E_2), CA (10^{-6}-10^{-4}M), T (10^{-7}-10^{-5}M), or DHT (10^{-6}M) prevented the stimulation of aromatase activity by Dex. E did not prevent the stimulatory effect of Dex. To examine the effect of CA, T, and DHT on the Dex-induced stimulation of aromatase activity, the ability to compete for binding to the glucocorticoid receptor (GR) was investigated. CA competed with (^{3}H)Dex for binding to GR and a 50% reduction of (^{3}H)Dex binding was obtained at 10^{-6}M, whereas both T and DHT were not the inhibitor of glucocorticoid binding to the receptor. On the other hand, when cells were preincubated with $(Bu)_2$c-AMP and CA, T, DHT, or E_2, the stimulatory effect of $(Bu)_2$c-AMP was not affected. In summary: 1) aromatase activity in FF can be induced by Dex or $(Bu)_2$c-AMP 2) Induction of aromatase activity by Dex is prevented by CA competing for binding to GR but other mechanism exists in prevention by T and DHT, and 3) Induction of aromatase activity by $(Bu)_2$c-AMP is not affected by CA, T, or DHT.