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143 *IN VITRO* METABOLIC STUDIES OF TESTICULAR TISSUE IN FAMILIAL MALE PSEUDOHERMAPHRODITISM DUE TO 17-KETOREDUCTASE DEFICIENCY.

Kinetic analyses of progesterone and androstenedione metabolism were performed in testicular tissue of 5 male pseudohermaphrodites (MPH) with familial 17-ketoreductase deficiency previously described (J. Steroid Biochem., 19:663-674, 1983), and compared to 2 normal controls. The age of the patients ranged between 3-3/4 and 35 years. Testicular tissue was obtained during surgery for male genitoplasty in 4 cases, and during castration in one individual, who had been reared unequivocally as a female. While the testicular tissue of the 2 prepubertal MPH metabolized progesterone to androstenedione only, and that in a limited extent, the 3 postpubertal MPH metabolized progesterone to 16∝- and 16ß-hydroxyprogesterone (16-OHP), 17-hydroxyprogesterone, androstenedione and to testosterone. These MPH metabolized androstenedione to testosterone as well, to a comparable extent as the controls. The Michaelis constant of these reactions was similar for the MPH and the controls. An 7.8 to 12.8-fold increase in the production of 16-OHP, and a 4.6 to 11.5-fold inhibition of the 17-hydroxylase was found in the testes of the MPH. The preference of androstenedione production by the testes of 2 MPH was examined using a equimolar concentration of [14C]-progesterone and [H3]-pregnenolone as substrates. While the flow of substrates in the normal testes was equal or slightly preferable through the Δ-4 pathway, a more than 8-times preference of the Δ-5 pathway was noted in the testes of the MPH. A large accumulation of DHEA was found upon omission of NAD, the 3ß-steroid dehydrogenase-isomerase cofactor, supporting the contention that in MPH androstenedione is mainly produced through the Δ-5 pathway. Further support for this was the finding that the 3H/14C ratio of androstenedione and testosterone produced from both substrates was 8-times higher in MPH than in controls. MPH due to 17-ketoreductase deficiency appears to be a mixture of complex metabolic aberrations in the androgen biosynthetic pathway.

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144 17,20-DESMOLASE DEFICIENCY IN A 8 MONTH OLD INFANT

During an uneventful pregnancy, prenatal sex determination of the fetus was performed on parental request. A 46,XY karyotype was found. At term, a phenotypic girl was born, and a second chromosome analysis confirmed the 46,XY constitution. There was a complete vagina, but on echography, gonads and uterus could not be seen with certainty. At age 8 months, FSH was 53 µU/mol, LH 3µU/l testosterone 1.1, and androstenedione 1.2 nmol/l. After hCG (5000 IU/m^2), there was no increment of testosterone. Urinary steroids before and after hCG and ACTH (gas chromatography on capillary column) suggest the presence of 17,20-desmolase deficiency: basal cortisol (total of THE, THF, alloTHF, cortolone, ß-cortolone 1.95 µmol/d) and progesterone metabolites (pregnanediol 0.14, pregnanetriol 0.2 µmol/d) were normal, but individual 17-ketosteroids (androsterone 0.42, etiocholanolone 0.14) low or undectable (DHEA). Thus, inspite of the common cytochrome P450, 17∝-hydroxylating activity was normal. Urinary 5ß/5∝ ratio was normal (0.33). In fibroblasts cultured from biopsy at labium majora androgen receptor binding was just below normal, but surprisingly the 5∝-reductase activity reduced (0.60 pmol/mg protein^{-1}h^{-1}; normal >1). This reduction appears to be secondary to the lack of the substrate testosterone during embryogenesis.
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145 ANALYSIS OF XX MALES AND XX TRUE HERMAPHRODITES, ARGUMENTS FOR A NON-Y TESTICULAR DETERMINING GENE.

9 patients having testicular tissue with 46,XX karyotype, aged 1 month to 16 years and followed through puberty, were studied. According to their phenotype they were 2 46,XX males with normal male phenotype and 7 with abnormal external genitalia (AG), among whom 4 with hypospadias and 3 with true hermaphroditism (TH). The endocrine data were identical in the three groups: testosterone normal during puberty, then decreased in adulthood, gonadotropin levels being already above the control values at mid-puberty. Biopsies were identical in the 2 subgroups of AG patients up to 5 years: no difference with controls, regardless of the ovarian part of the ovotestis ; after 8 years, germ cells disappeared and dysgenesis became obvious. In one patient, the ovarian zone of the gonad appeared only after complete serial cuts of the gonad removed. Southern analysis of Y-DNA sequences displayed the presence of Y-specific material in 46,XX classical males and the lack of the sequence in all patients with AG.
These findings, together with the knowlegde of familial cases of XX males with AG and TH allow to consider that XX males with AG and TH are the alternative expression of the same genetic defect and that a non-Y testis determining factor might be active in these patients in place of the testis determinants usually present on the Y chromosome, or on the X chromosome in XX classical males.

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146 PERIPHERAL AROMATASE ACTIVITY IN ANDROGEN INSENSITIVITY SYNDROMES.

Basal and androgen-stimulated aromatase activity was studied in genital skin fibroblasts (GSF) from normals (n = 18) and patients with androgen insensitivity syndrome (AIS; n = 8). Enzyme activity was determined from the release of [^{3}H] ^3H_2O following the conversion of [1ß-^{3}H] androstenedione ([1ß-^{3}H]-A) to oestrone. Using a substrate concentration 2-50 nM, saturation kinetic analysis yielded a Vmax (maximum velocity) of 184 ± 104.1 fmol/mg protein/hr (mean ± SD) and a kM (Michaelis-Menten constant) of 6.9 ± 2nM in normal GSF strains, and values of 186 ± 9.1 (Vmax) and 8.5 ± 2.6 (kM), respectively in AIS strains. Androgens increased aromatase activity in normal GSF strains. Pre-incubation with 10nM mibolerone (a synthetic androgen) for 48 hr produced a 4.2 - 38 fold increase in basal aromatase activity. The response in receptor-positive (n = 5) and receptor-deficient (n = 2) partial AIS strains was 12.4 - 23.5 and 1.1 - 2.4 fold, respectively. There was no stimulaton of aromatase in a receptor-negative complete AIS strain. The results provide evidence of a permissive role for androgens in the control of peripheral aromatase activity. The effect is androgen receptor mediated based on studies of GSF strains from AIS patients with a dysfunctional receptor, and may provide an additional in vitro qualitative marker of androgen responsiveness.

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147 IMPAIRED SEXUAL DEVELOPMENT DUE TO TESTICULAR DYSFUNCTION AND TARGET ORGAN INSENSITIVITY. DIFFERENTIATION BY A NEW ANDROGEN SENSITIVITY TEST.

Regular testicular function and responsiveness of target organs to testicular androgens are prerequisites of normal male sexual development. Impairment of either function can cause varying degrees of incomplete masculinization. A pilot study had revealed a different SHBG-reaction to anabolic steroids in patients with the testicular feminization syndrome (ESPE'87, Abstr.No.160). We now investigated the effect of Stanozolol and Oxandrolone (3 days 0.2 mg/kg *per os*) on SHBG, CBG, TBG, albumin, total protein, testosterone, estradiol, thyroxin, cortisol, LH and FSH in 4 patients with impaired testicular function (gonadal dysgenesis, 17ß-hydroxysteroid-dehydrogenase defect), 4 patients with target organ insensitivity (partial and complete androgen resistance), and in 20 controls. In testicular dysfunction and in the controls SHBG-levels decreased significantly ($p<0.001$) down to a minimum of 51.6 % ± 6.0 (SD) (Stanozolol) and 66.2 ± 10.3 (SD) (Oxandrolone) of the initial values at days 5,6,7, or 8 after initiation of the test, while in partial androgen resistance the decrease was moderate (81% and 89%), and absent in complete testicular feminization. Differences in all other parameters were not statistically significant. Conclusion: 1.) The SHBG-decrease induced by short term anabolic steroid application is a specific reaction. 2.) This reaction requires regular target tissue sensitivity. 3.) It can be used for the differentiation of testicular dysfunction syndromes from androgen insensitivity syndromes.

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148 MALE PSEUDOHERMAPHRODITISM (MPH) DUE TO BOTH TESTICULAR 17-KETOREDUCTASE AND PARTIAL ADRENAL 3ß-HYDROXYSTEROID-DEHYDROGENASE (3ß-HSD) DEFICIENCY.

A patient with female phenotype at birth, a history of bilateral inguinal hernia and karyotype 46,XY was considered to have MPH due to testicular feminization syndrome until 13 years of age when she developed clitoromegaly (3.5 x 1.8 cm), a deep-pitched voice and breast development. The blind vaginal pouch ended at 3 cm and no prostatic tissue was palpable at rectal examination. Peripheral serum steroid analyses revealed markedly elevated levels of androstenedione (Δ4)(4.20 ng/ml) and DHEA (9.50 ng/ml) and moderately raised concentrations of testosterone (T)(1.72 ng/ml). The finding of an elevated Δ4/T ratio was consistent with 17-ketoreductase deficiency. Spermatic vein blood with an even higher Δ4/T ratio (22:1) suggested testicular origin of the increased secretion of Δ4. Neither HCG nor ACTH administration caused further significant increases of Δ4 or T, whereas DHEA showed a 3-fold rise after ACTH. The gaschromatographic profile of urinary steroid metabolites indicated predominance of 5α-reduced steroids. Surgical exploration for gonadectomy revealed an inguinal right and an abdominal left testicle. No remnants of the Mullerian ducts could be found. Histologically, both gonads showed epididymes and vasa deferentia as well as atrophic testicular tissue with Leydig cell hyperplasia, Sertoli cells and few spermatogonia. Genital skin fibroblasts exhibited normal androgen binding and 5α-reductase activity. After gonadectomy Δ4 and T levels fell to normal; DHEA, however, remained strikingly high (17 ng/ml) suggesting a coexisting partial adrenal 3ß-HSD deficiency.