11

P.C. WHITE*,M.I.NEW and BO DUPONT*, Sloan-Kettering Institute and Cornell Med. Ctr., New York, NY, USA

HLA-LINKED CONGENITAL ADRENAL HYPERPLASIA RESULTS FROM A DEFECTIVE GENE ENCODING A CYTOCHROME P-450.

Congenital adrenal hyperplasia due to 21-hydroxylase (21-OH) deficiency is HLA-linked. The haplotype HLA-(A3);Bw47;DR7 is strongly associated with 21-OH deficiency and always carries a null allele at the complement C4A (Rodgers) locus. It seemed likely that this haplotype carries a deletion encompassing both the C4A and 21-OH loci. We hypothesized that the HLA-linked defect involved a structural gene for the adrenal microsomal cytochrome P-450 specific for steroid 21-hydroxylation. We isolated a plasmid with a 520 bp bovine adrenal cDNA insert encoding the middle third of the P-450 peptide [White et al. PNAS April 1984]. When human DNA was digested with Taq I restriction endonuclease and hybridized with the cDNA probe, DNA from 13 unrelated normal individuals yielded two hybridizing bands of equal intensity at 3.7 and 3.2 kb. The upper band was not present in DNA from a patient homozygous for Bw47. DNA from six unrelated patients heterozygous for Bw47 yielded, in five, diminished relative intensity of the upper band consistent with a heterozygous deletion, and complete disappearance of the upper band in one (X^2=15.9, p=.0001). Linkage of this polymorphism to HLA was examined in the families of several of these patients; the lod score exceeds 3.0 at a recombinant fraction of 0.0. Thus 21-OH deficiency sometimes results from the deletion of a gene and sometimes, presumably, from smaller mutations. This gene is probably located very near the C4A gene encoding the 4th component of complement.

12

U. KUHNLE*, HG. DÖRR*, F. BIDLINGMAIER, D. KNORR, MI. NEW, S. ULICK*. Universitätskinderklinik, 8000 München 2, WGermany, Cornell Medical Center, NY, NY 10021, Veterans Administration Hospital, Bronx, NY 10468.

EVIDENCE FOR A NATURALLY OCCURING MINERALOCORTICOIDANTAGONIST IN CONGENITAL ADRENAL HYPERPLASIA (CAH).

The existence of a salt-excreting factor in CAH due to a 21-hydroxylase defect has been postulated for many years.However extensive investigations sofar were unable to isolate such a factor.Our demonstration of a salt-excreting factor in CAH utilized direct and indirect measurements of the mineralocorticoidactivity.The direct method was based on the ability of mineralocorticoid agonists and antagonists to displace aldosterone in a mineralocorticoid radioreceptor assay.The indirect method measured the separate contributions of the principal mineralocorticoid agonists:aldosterone, 11-deoxycorticosterone,corticosterone and cortisol as determined from their relative receptor affinity. In controls there was good agreement between these two measurements.However,when plasma extracts from patients with CAH were assayed, the direct method showed almost twice as much activity as the indirect method. This difference in mineralocorticoid receptor activity was observed in both the salt-wasting and the non-salt-wasting form and was suppressed by dexamethasone and restored by ACTH. We propose that this difference results from the presence of a mineralocorticoid antagonist which competes with the known agonists for the mineralocorticoid receptor. In salt-wasting CAH this antagonist may contribute to salt-wasting symptoms;in the simple-virilizing form this antagonist may cause hyperreninemia and hyperaldosteronism.

13

P J SMAIL*, J S D WINTER* (Intr. by C S SMITH) Department of Paediatrics, University of Manitoba, Winnipeg, Manitoba, Canada and University of Aberdeen, Scotland, UK.

Effects of ACTH and oestradiol on steroid production by cultured adrenal cells from an anencephalic foetus and from normal adults.

Dispersed adrenal cells from a 16 week anencephalic foetus, 7 foetuses with intact pituitaries and 3 adult subjects at the time of renal transplantation were maintained in tissue culture for 6 days and the steroidogenic responses to ACTH ($0-13^3$ pg/ml) with or without added oestradiol ($0-10^4$ ng/ml) were evaluated. The anencephalic cells showed a delayed response to ACTH but by the fifth day production of cortisol (1 µg per 10^5 cells per day), dehydroepiandrosterone (DHA) and DHA-sulphate was similar to that in the other cultured foetal adrenal cells. The addition of oestradiol caused dose-related inhibition of cortisol production and concomitant increase in DHA and DHA-sulphate production. The adult adrenal cells in the presence of ACTH showed a much higher cortisol-DHA secretion ratio (on average 60-hold higher) but the addition of oestradiol markedly reduced this ratio as in foetal cells. These data support the suggestion that the relative 3β-hydroxysteroid dehydrogenase deficiency characteristic of the foetus is not an intrinsic property of foetal adrenal cells but is imposed by the combined effects of ACTH and steroids within the foetal environment (such as oestradiol).

14

H.G.DÖRR*, U.KUHNLE*, F.BIDLINGMAIER and D.KNORR Div of Paediatric Endocrinology, Dept of Paediatrics University Hospital of Munich, Germany

Etomidate (E): a potent inhibitor of adrenocortical 11 ß-hydroxylase activity

Increased mortality with long-term E infusions in intensive care patients has been reported and evidence is accumulating that E causes reversible adrenocortical suppression with decreased serum cortisol levels. A direct inhibition of adrenal steroidogenesis has been suggested, but so far data on the mechanism of this action are lacking. A 6 1/2 year old boy with convulsions due to encephalitis was treated with constant E infusions (1 - 2 mg/kg/h). Prior to E administration, adrenocortical function was normal as tested by an ACTH-test. 5 days after institution of therapy and 14 days after discontinuation, plasma levels of 11-deoxycorticosterone (DOC), corticosterone (B), 11-deoxycortisol (S) and cortisol (F) were measured by RIAs after Sephadex LH-20 chromatography. During E therapy DOC and S levels were highly elevated, while B was in the lower normal range and F was markedly decreased. Likewise the ratios of B/DOC and F/S, which reflect adrenocortical 11 ß-hydroxylase activity, were extremely decreased (B/DOC:0.06 vs 27 in controls; F/S:0.009 vs 230 in controls). After discontinuation of therapy, the absolute values as well as the ratios returned to normal. These results show, that E is a potent inhibitor of adrenal 11 ß-hydroxylation.Increased precaution seems to be necessary when this drug is used in patients who are under considerable amount of stress.

15

M.G.FOREST and J.BERTRAND. INSERM-U.34, Hôpital Debrousse, 69322 Lyon Cedex 05. France.

Δ^4-Androstenedione (Δ^4), a biochemical marker of 11β-hydroxylase deficiency (11β-OHD).

Two female newborns (N°1 & 2), a 13-yr girl (N°3) (stages II-IV) and a 2-yr boy (N°4) were studied. 11β-OHD was ascertained on elevated levels of plasma 11-deoxycortisol (S) or urinary THS. Plasma levels (in nmol/l) of Δ^4, testosterone (T)and 17α-OH-progesterone (OHP) were followed before, at, and during replacement therapy (Rx),after ACTH (1 mg/m^2/dx3) or Dex (2 mg/dx8). Before Rx, Δ^4 (33 to 297) was always higher than OHP. The molar Δ^4/OHP ratio was constantly high (2.7 ± 1.1;n=10), in contrast to patients with 21-OHD in whom this ratio is ≃0.03 (0.009 to 0.5). In case N°1, OHP was subnormal at 2-27 days of age; 11β-OHD was suspected only on a high (2-4)Δ^4/OHP ratio; after ACTH,Δ^4 rose drastically (34.4 to 144), more than OHP(8.6 to 66) and Δ^4/OHP remained similar (2.2).Under Dex (case N°4),OHP dropped to normal within 24 h, while Δ^4 decreased slowly and remained abnormally high (5.9) on day 8,thus a stricking rise in Δ^4/OHP (2.2 to 18-32) occured during the test. The same pattern was seen in all cases at initiation of Rx. Diurnal variations were larger for OHP(4-10 fold) than for Δ^4(2-3 fold). Under long-term Rx(2-10 yr),despite low normal OHP, T was still above normal when Δ^4/OHP was >1. In conclusion, 1)there is a selective accumulation of Δ^4in 11β-OHD which is likely due to the impaired metabolism of Δ^4into 11β-OH Δ^4(normal inactivation pathway) providing an increased substrat for peripheral T conversion. 2)Δ^4/OHP is as useful as plasma S,and probably more sensitive,in monitoring Rx and should ideally be < 1.

16

S. PANG*, M. LOO*, M.S. POLLACK*, B. DUPONT*, O. GREEN*, G. CLAYTON*, M.I. NEW. The New York Hosp-Cornell Med Ctr and Memorial Sloan-Kettering Cancer Ctr, New York, NY 10021; Children's Mem Hosp, Chicago, IL 60614; Baylor Univ Col Med, Houston, TX.

Pitfalls of prenatal diagnosis of 21-hydroxylase deficiency (21-OH def) congenital adrenal hyperplasia (CAH).

Hormonal measurements and HLA genotyping of amniotic fluid at midgestation correctly predicted the postnatal dx of CAH in 26 of 29 fetuses at risk for CAH. Of these 26, 6 were predicted to have classical 21-OH def based on elevated amniotic fluid 17-hydroxyprogesterone (17-P) and Δ4-androstenedione (A). These 6 fetuses and their index cases were ultimately proven to have salt-wasting classical 21-OH def. Of 3 HLA typed, genotype was identical to the index case. Normal amniotic fluid 17-P and A in the remaining 20 predicted fetuses unaffected with classical CAH, and these patients have been clinically asymptomatic to date or biochemically proven not to be affected with classical or nonclassical CAH. Of the 20 fetuses, 6 were HLA typed and predicted to be homozygous unaffected or heterozygous. However, in 3 of the 29 fetuses, prenatal diagnosis was incorrect. In one, the fetus was predicted to have CAH based on HLA identity to the index case, but amniotic fluid 17-P and A were normal and the fetus was normal. The index case of this family did not have CAH but was a normal child. Thus, amniotic fluid hormone levels accurately predicted a normal fetus while HLA typing was not relevant in prenatal dx because the index case was unaffected. The second fetus was predicted to be a carrier on the basis of HLA typing and normal amniotic fluid 17-P and A. However, during infancy the female infant was shown to have nonclassical CAH and be HLA identical to the index case. The index case in this family, presumed to have classical CAH, was later diagnosed to have nonclassical CAH. In the third case, the fetus was predicted to be a heterozygote by HLA genotyping and to be unaffected by hormonal measurement. Postnatally, at age 2 7/12 yrs, the male child was shown to have non-salt-losing classical CAH and to be HLA identical to the index case who also has non-salt-losing CAH.

These data demonstrate that in salt-losing classical CAH, prenatal dx of the homozygous affected fetus is reliable by the measurement of amniotic fluid 17-P and A. In nonclassical or non-salt-losing classical CAH, amniotic fluid 17-P and A are in the normal range, thus prenatal dx is not possible by hormonal evaluation. Both hormonal and HLA studies of fetal amniotic fluid, as well as correct dx of the specific form of 21-OH def and HLA studies of the index case, are essential in diagnosing the specific form of 21-OH def CAH in the fetus.