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OPPOSITE EFFECT OF PROTEIN KINASE C ACTIVATION ON STEROIDOGENIC ENZYME GENE EXPRESSION IN HUMAN CHORIOCARCINOMA CELLS AND IN ADRENOCORTICAL CELLS.

The cholesterol side-chain cleavage enzyme (P450scc) is the rate-limiting and hormonally regulated step in steroid hormone synthesis. Cyclic AMP (cAMP) is thought to be the main second messenger regulating steroid hormone synthesis in all steroid producing organs. We studied the effects of 12-O-tetradecanoyl phorbol 13-acetate (TPA), an activator of protein kinase C, on P450scc mRNA levels and steroid production in cultured human choriocarcinoma (JEG-3), fetal adrenal and adult adrenal cells. In JEG-3 cells TPA (up to 100 ng/ml) increased P450scc mRNA accumulation 230 % ($p < 0.001$) and progesterone secretion 320 % ($p < 0.01$) simultaneously. In cultured human fetal and adult adrenal cells TPA decreased ACTH-stimulated P450scc and P450c17 (17-hydroxylase/17,20 lyase) mRNA levels 30-80 % ($p < 0.01$). At the same time cortisol and corticosterone secretion decreased at least 50 % ($p < 0.01$). The data show that protein kinase C activation leads to stimulation of steroidogenesis in choriocarcinoma cells, but to inhibition in adrenal cells. Adenylate cyclase activation leads to increase in steroidogenesis in both cell types. It will be of interest to see if growth factors causing protein kinase C activation, will cause similar changes in steroidogenesis as TPA does.

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SEVERE HYPOALDOSTERONISM DUE TO CORTICOSTERONE METHYL OXIDASE TYPE II (CMO II) DEFICIENCY IN 2 BOYS: METABOLIC AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC-MS) STUDIES

Infection-triggered life-threatening salt loss and hyperkalemia developed in 2 male infants with dystrophy, inappropiately low plasma aldosterone concentrations and elevated plasma renin activity. Sodium supplementation but not short-term high dose oral 9α-fluorcortisol (FF) did revert hyponatremia in one boy (Pat.A) The other boy (Pat.B) is growing normally on a high sodium diet and oral FF (0.1 mg/d). Diagnosis of a defective terminal step of aldosterone biosynthesis was confirmed by measuring urinary excretion of specific mineralocorticoid metabolites:

Pat.	CA (y;mo)	THB µg/24 h (nl. range)	18-OH-THA µg/24 h	THB + a-THB / C-M (nl. ratio)
A	4;9	640 (0-307)	1249(nl.:not detectable)	0.66 (0.26)
B	2;7	160 (0-225)	127(nl.:not detectable)	0.44 (0.18-0.22)

Unknown steroids as candidates responsible for the salt loss could not be identified by GC-MS. In conclusion, GC is mandatory in all cases of unexplained salt loss in infancy and childhood. Short-term response to exogenous mineralocorticoids may not be diagnostic in distinguishing pseudohypoaldosteronism from defects in mineralocorticoid synthesis. (THA: 11-dehydrotetrahydrocorticosterone, a-THB: allo-tetrahydrocorticosterone, C-M: cortisol metabolites)

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ADRENAL RESPONSE DURING THE GRAFT-VERSUS-HOST (GVH) IN AN AVIAN MODEL

The GVH is a frequent complication of bone marrow transplantation (BMT) leading to a multi organ immune-mediated pathology. Its endocrine effects are difficlt to analyze in human pathology as BMT requires multiple regimen preparation frequently including radiotherapy. An avian model has been developped to more precisely investigate the endocrine complications of GVH. GVH was elicited on 9-day old chicken embryos by adult histo compatible spleen graft on the chorio allantoic membrane. The intensity of GVH was evaluated by the alteration of the embryo spleen. Adrenals from control and allografted animals were perifused (Endotronics, USA) during 2 hours and fractions collected every 10 minutes. In the controls, corticosterone production was constant during the perifusion period. Maximal corticosterone response occurs within 10-20 minutes after the injection of 1-24 ACTH. The optimal ACTH dose was 0.5 ug/ml. The adrenals of GVH animals shown basal secretion (6.0 ± 2.2 ng/ml/100 mg/10 mn) similar to the control. The maximal response to ACTH was constantly delayed (peak at the 20-30 minutes fraction) and significantly reduced compared to the control (400 % vs 900 % of the basal values). The present data favor adrenal resistance to ACTH. Such a resistance has been previously found in the thyroid on the same model. The exact mechanisms of this is presently under closer investigation.

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HUMAN CORTICOTROPIN-RELEASING FACTOR (CRF) AND ACTH TEST IN PREMATURE PUBARCHE (PP).

Serum and plasma concentrations of ACTH, beta-endorphin (B-EN), dehydroepiandrosterone (DEA), dehydroepiandrosterone sulfate (DS), 17-hydroxyprogesterone (17-OHP), androstenedione (D_4) and cortisol (F) were measured before and after i.v. administration of 1µg/Kg of CRF (CRF 1-41, Nova-Biochem, CH) in 9 patients with PP (8 F, 1 M, mean age 6.9±1.2yrs, bone age 7.6±1.3yrs) and in 9 children with Tanner stages II-III (CC) (5 F, 4 M, mean age 11.5±1.2yrs, bone age 11.2±1.5yrs). The same hormones were measured before and after i.v. injection of 0.25 mg. ACTH (Synacten) in the same subjects. The results obtained can be summarized as follows: CRF test; 1) No differences in baseline and peak plasma levels of ACTH and B-EN between the 2 groups; 2) Peak serum DEA levels, ΔDEA values significantly lower in PP than in CC (peaks 1.77±0.9 v.s. 3.12±1.02 ng/ml, $p < 0.025$, x±SD; Δ 0.12±0.47 v.s. 1.18±0.87 $p < 0.025$); 3) Peak 17-OHP serum levels significantly higher in PP compared to CC (1.23±0.24 v.s. 0.81±0.3 ng/ml, $p < 0.025$); 4) Peak D_4/peak 17-OHP ratio significantly lower in PP than in CC (0.57±0.17 v.s. 1.26±0.46, $p < 0.01$). ACTH test: baseline and peak serum levels of all measured hormones similar in the 2 groups. In conclusion, different androgen responses to CRF administration seem to be independent from ACTH and B-EN secretion. In PP, under CRF stimulation, the apparent C_{17}-20 lyase activity does not appear to be functioning as in children with the same degree of pubic hair development but with associated gonadarche. Whether this difference in enzyme efficiency is due to the action of a hypothalamic-pituitary factor other than ACTH and B-EN, or to intrinsic adrenal changes is still unclear.

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ADRENOCORTICAL INSUFFICIENCY ASSOCIATED WITH ACHALASIA AND ALACRIMA: VARIABILITY OF CLINICAL FINDINGS IN TWO CASES.

After the 4 patients reported by Allgrove in 1978, only few cases of the ACTH insensitivity, achalasia and alacrima syndrome have been described. Some clinical aspects of the disorder and its pathogenesis has not yet been clarified. We report 2 more patients to confirm the clinical entity of the syndrome. Case 1 male patient, developed achalasia at age 2.5 yrs (Heller's myotomy was carried out) and after 1.5 yrs glucocorticoid deficiency (skin pigmentation, convulsions with hypoglycaemia): ACTH > 850 pg/ml, no response to ACTH test, normal aldosterone and PRA. Eyes apparently normal, but evidence of impaired tear production after Schiller test. Other features: muscular hypotrophy and hypotonia, foot orthopedic abnormalities. No findings in parents. Case 2 female with alacrima since birth; at age 3.5 diagnosis of adrenal deficiency was made (weakness, skin pigmentation, convulsions with hypoglycaemia): ACTH levels ranging from 229 to 427 pg/ml, undetectable cortisol levels, urinary aldosterone less than normal. She has sligth dysphagia without radiological signs of achalasia. Isolated findings within family: alacrima in maternal grand - mother, elevated ACTH levels in mother's sister. Conclusions: our cases show the clinical variability of this syndrome; the presence of isolated achalasia, adrenocortical insufficiency or alacrima in a child warrants evaluation for the other components.

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PROTECTIVE EFFECT OF ATRIAL NATRIURETIC PEPTIDE (ANP) ON CELLS DAMAGED BY HYPOXIA OR OXYGEN RADICALS.

Recent reports[1,2] show that ANP attenuates acute ischemic renal failure (ARF) in vivo and in isolated perfused kidney. ANP is a potent renal vasodilator, and the protective effect of ANP was shown to be mediated by improving hemodynamics. To determine if ANP also has a protective effect at the cellular level, studies were performed in hepatocyte cell cultures, also known to respond strongly to ANP. Cells were exposed to A) 0.5% O_2 for 4 h and reoxygenation at 20% O_2 for 20 h or B) hypochlorous acid (100 µM) for 1 h. Both procedures are known to lead to the production of free radical metabolites, which cause cell damage. In response, hepatocytes showed increased membrane lesions (assessed by release of serum glutamate transaminase, bleb formation and non-exclusion of trypan blue) and proteolysis (assessed by the decrease of trichloroacetic acid-precipitable [^{14}C]valine-labeled peptides). ANP (0.01, 0.1 and 1 µM) given at any time during the experiment, protected the cells against further membrane damage and proteolysis or, at least, attenuated these processes. This cytoprotective effect of ANP was paralleled by an up to 3-fold increase in cellular cGMP content. Both cytoprotection and the increase in cGMP could be blocked by pertussis toxin, which strongly suggests that the ANP-particulate guanylate cyclase response is mediated by a G-protein in hepatocytes. Sodium nitroprusside, which also increased the cells cGMP content via cytosolic guanylate cyclase, had a cytoprotective effect similar to ANP. These results show that the beneficial effect of ANP in ARF may not only be hemodynamically mediated but can also be the result of cytoprotective properties. Furthermore these results stress the possible importance of cGMP and cGMP-mediated response in cytoprotection.

)[1,])[2] J.Clin.Invest. 80(1987), 698, 1232