135 CHARACTERISTICS OF PROTEIN KINASE C MEDIATED PITUITARY ACTH RELEASE. Douglas O. Sobel, Depts of Peds and Med, Naval Hospital, Bethesda, and Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA.

Protein kinase C is a proposed mediator of pituitary ACTH secretion. To investigate this role of protein kinase C, we studied the effect of 12-O-tetradecanoylphorbol-13-acetate (TPA), an inducer of protein kinase C activation, on in vitro pituitary ACTH release. Normal rat anterior pituitaries were enzymatically dispersed to monolayer culture. Dishes with 3.5×10^5 cells were incubated 3 days, washed, and incubated in test media for 3.5 hours. TPA stimulated ACTH(RIA) release in a dose related fashion with an ED_{50} of 0.7 ng/ml and a maximal stimulation of 900% over unstimulated cells. Increasing the concentration of culture media calcium from 0 to 1 mM, 3mM and 6 mM led to a significant increase of TPA induced ACTH release from 4.80 to 6.79, 8.41, and 8.62 ng/dish respectively. Nifedipine (10 μM), a membrane calcium channel blocker, and penfluridol (10 μM), a calcium calmodulin antagonist, inhibited TPA (1 ng/ml) mediated ACTH release by 21% and 31% respectively ($p < 0.01$). The addition of PGE_2 (0.1 μM), an inhibitor of CRF mediated ACTH release, caused a 33% decrease of TPA stimulated ACTH release. TPA (100 μg/ml) significantly increased intracellular PGE_2(RIA) and PGE_2 released into culture media by 178% and 172% respectively, over unstimulated cells. In conclusion, TPA stimulated ACTH release in vitro appears to be dependent on extracellular calcium, membrane calcium influx, and calcium calmodulin activation. Also, TPA stimulates pituitary PGE_2 synthesis and exogenously administrated PGE_2 inhibits TPA induced ACTH release.

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THE DEVELOPMENT OF IMMUNOREACTIVE CORTICOTROPIN-RELEASING FACTOR (IR-CRF) IN THE HYPOTHALAMUS, PANCREAS AND GASTROINTESTINAL TRACT OF NEONATAL RATS.

Using a specific RIA technique for rat CRF, we have determined the IR-CRF concentrations in acid extracts of hypothalamic, pancreatic and gastrointestinal tissues in fasting and fed immature rats (1 day before birth, 0, 1, 3, 7, 14, 21, 28 and 42 days after birth). IR-CRF from these tissue extracts gave dilution curves that were parallel to the rat CRF standard curve and co-eluted with synthetic rat CRF on Sephadex G-50 gel chromatography. The mean hypothalamic IR-CRF concentration was 7.4±4.6ng/g wet weight of tissue 1 day before birth and remained almost constant during the first week of life. Then, it increased gradually about 2-fold up to day 42. The mean IR-CRF concentrations in the pancreas and jejunum were very low before birth, but they increased abruptly about four times higher than that in the hypothalamus immediately after birth. Thereafter, they declined progressively with age. On the other hand, the mean IR-CRF concentrations in the gastric antrum and duodenum were much lower than that in the hypothalamus throughout maturation. No significant difference was seen between fasting and fed rats in any age group. Conclusion; The mean IR-CRF concentrations in the pancreas and jejunum had the very high peak while that in the hypothalamus remained constant in early neonatal period. These findings suggest that CRF in the pancreas and jejunum may play a particular role in neonatal rats.

137 DEVELOPMENT AND APPLICATION OF A HIGHLY SPECIFIC SOLID-PHASE RIA FOR ALPHA-MSH: LACK OF THE HORMONE IN PREGNANTS AND NEWBORNS. Alex N. Eberle, Jürg Girard, Joyce B. Baumann and Ernst Bürgisser. University Children's Hospital and University Hospital, Department of Research, Basle, Switzerland.

Alpha-MSH has been reported to occur in the plasma of pregnant women (Nature 273: 163, 1978). Since it appeared that the RIA used for those studies was not sufficiently sensitive and specific, we have developed a new solid-phase RIA for alpha-MSH with a sensitivity of 2-4 pg per ml of plasma and a negligible cross-reaction with other hormones (ACTH: <0.1%). Goats were immunized with chemically defined carrier protein-MSH complexes and the antibodies covalently coupled to a non-sedimenting acrylamide polymer. The new antibody-carrier complex allows an immune extraction from samples containing 30-50% plasma before addition of the tracer, so that any 'plasma effects' could be avoided. Applying this RIA to 136 samples from the 6th week of pregnancy till term and to cord blood samples showed clearly that they did not contain detectable alpha-MSH in the circulation. In contrast, alpha-MSH was shown to be present in fetal life, supporting the hypothesis of its trophic action on the adrenal. It seems therefore that the presence of MSH is restricted to the fetus and is independent from the mother.

138 OUTCOME OF CHILDHOOD HYPERINSULINISM
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We have reviewed the outcome in 34 patients seen between 1964 and 1984 with hypoglycaemia due to hyperinsulinism, all but 4 of whom presented in the first year of life. Twelve of the 15 cases who presented before 1 month of age underwent pancreatectomy. One case died postoperatively and 3 have been lost to follow up. Six cases are making normal developmental progress, one is retarded and one has convulsions. Fifteen cases presented between the ages of 2 and 7 months; 10 out of 13 showed leucine sensitivity. Only 2 out of these cases required surgery and the others were treated with diazoxide or a low leucine diet. Of the 16 cases treated medically, one with cerebral palsy died at 1 year and 1 has been lost to follow up. The remainder have been followed for 3.5-16 yrs. Treatment was stopped in 11 cases after 2.7-9 yrs (median 4.75 yr) without return of hypoglycaemia although one case is still leucine sensitive. Of this group, 8 are of normal intelligence, 6 are retarded and 3 are borderline. The children who present in later childhood (2.5-10 years) were all treated surgically and the outcome has been excellent.

139 IMPAIRED GLUCAGON COUNTERREGULATION IN CHILDREN WITH INSULIN-DEPENDENT DIABETES (IDD). Carol J. Singer-Granick, Dorothy J. Becker, Allan L. Drash, University of Pittsburgh, Dept. of Pediatrics, Pittsburgh, PA USA

Intravenous insulin (0.3-0.5 u/kg) was given over 2 mins to 36 pts with IDD, duration 1.7-16.7 yrs ($\bar{x}$ 8.2±4.2) aged 10.9-18.4 yrs ($\bar{x}$ 15.8±1.8). Plasma glucagon (IRG) responses were compared to those of 6 endocrinologically normal children with short stature (C) aged 13.2±3.2 yrs. Antibodies to IRG were found in 3 IDD pts who had relatively poor recovery from hypoglycemia. Increments from baseline (Δ) & peak IRG in the other 33 IDD pts were markedly lower than in C (80±67 vs 354±79 pg/ml, p=<.001, 211±97 vs 407±87, p<.001, resp). IRG response did not correlate with age, duration of IDD, free insulin (FI) levels (n=25), or % insulin antibody binding (IAb) (n=10). Also Δ IRG did not correlate with plasma glucose (G), nadir, % drop, or G recovery from nadir. This G recovery was impaired compared to C (P<.001) & did not correlate with FI levels or % IAb. 10 pts were studied after both insulin withdrawal & 3 days of intensive insulin Rx with no significant difference in IRG response. During intensive Rx there was a +ve correlation between IRG Δ, & G recovery from hypoglycemia (P<.001). Adequate IRG reserve was demonstrated during insulin withdrawal by a greater IRG response to a mixed meal than insulin (Δ 156±80 vs 55±57 pg/ml; P<.001); however, there was a +ve correlation between the IRG peaks after these 2 stimuli (r=.93, P<.001) suggesting some impairment of α cell function. The significance of this must be related to multiple other counterregulatory abnormalities, but IRG deficit appears to be a primary factor during intensive Rx.

140 SYNDROME OF ACANTHOSIS NIGRICANS, POLYCYSTIC OVARIES, AND INSULIN RESISTANCE: A COMMON ENDOCRINOPATHY IN ADOLESCENTS. Geoffrey P. Redmond, Gita Gidwani, James Taylor, Cleveland Clinic Foundation, Depts. of Endocrinology, Gynecology, Pediatrics, and Dermatology, Cleveland, OH USA

Although the association of acanthosis nigricans (ACN), with abnormalities of carbohydrate metabolism and ovarian function has recently received attention in the medical literature, the impression remains that this is a rare condition. Over the last 2 years, we have seen 21 patients with this syndrome. 73% were younger than 20 years. Amenorrhea was the most common presenting complaint but others came because of hirsuitism, obesity, diabetes or the skin changes.

Initial results on 10 patients indicate that 2 had 1° amenorrhea, 6 had 2° oligo-or amenorrhea, 2 continued to menstruate. 8 were caucasion and 2 were black. Age at diagnosis ranged from 14 and 3/12 to 20 years. ACN had been present between 2 months and 4½ years but many patients were unaware that they had the condition. 7 of the 10 patients had one or more elevated androgen levels (free or total testosterone, androstenedione or DHEA-S); 4 of 10 had abnormal glucose tolerance but 8 had elevated 1 hr insulin levels which ranged from 100 to 720 μU/ml. Treatment remains problematic but weight loss or glyburide improves glucose tolerance in some. CONCLUSIONS: 1) The syndrome of ACN-PCO is common in adolescents, 2) Most patients with ACN have elevated androgens, and 3) Most have abnormal insulin action.