

35

K.Pesonen*, L.Viinikka*, A.L.Mattila*, A.Koskimies*, J.Perheentupa
Children's Hospital, University of Helsinki,
Helsinki, Finland
EPIDERMAL GROWTH FACTOR (EGF) IN HUMAN BODY FLUIDS

To elucidate the distribution and nature of EGF in human body fluids, we measured immunoreactive EGF (IR-EGF) by a RIA or a (more sensitive) time-resolved immunofluorometric assay, and evaluated its size heterogeneity by size exclusion high performance liquid chromatography. The mean concentration was 80ng/ml in urine, 65ng/ml in milk, 50ng/ml in seminal plasma, 25ng/ml in armpit sweat, 1.0 ng/ml in breast sweat, 3ng/ml in saliva, 1.5ng/ml in tears, and 0.3ng/ml in gastric juice and third trimester amniotic fluid. All the fluids contained the standard size 6.2 kilodalton (6.2K) IR-EGF molecule. It was the only component present in armpit sweat and gastric juice. Additionally, we observed the total of 5 different molecular sizes of IR-EGF, ranging in approximate molecular weight from 400K to 15K. The standard form made up >90% of the total IR-EGF in all the fluids except the amniotic fluid, where its proportion was 71%. A >400K component was present in milk and amniotic fluid, a 300K component in urine and seminal plasma, a 200K component in breast sweat, a 80K component in seminal plasma, saliva and tears and a 15K component in urine, seminal plasma, and the amniotic fluid. It remains to be studied whether the larger than standard forms of IR-EGF represent different cleavage products of a common precursor, different tissue specific products, aggregates of EGF, EGF bound to different proteins, or several of these.

36

J. Weil,* R. Gerzer,* D. Knorr, C. Döhlemann,* T. Strom,* R. Lang,* H. Dörr* F. Bidlingmaier. Dept. of Pediatrics, Univ. of Munich, and Dept. of Clinical Biochemistry, University of Bonn, FRG.
PLASMA ATRIAL-NATRIURETIC PEPTIDE (ANP) AND cGMP LEVELS IN CHILDREN. cGMP AS MARKER FOR THE EFFECT OF ANP ON TARGET TISSUES.

The apparent inability of elevated atrial-natriuretic peptide (ANP) levels to cause natriuresis and diuresis in children with heart failure may be related to a reduced response of target organs to ANP. The cellular effects of ANP seem to be mediated by cGMP as the second messenger of ANP. Simultaneous measurement of ANP and cGMP has been shown to be a useful tool to study the cellular response to ANP. Therefore, plasma ANP and cGMP levels were measured by radioimmunoassay in 168 healthy children and in 47 children with congenital cardiac diseases. In healthy children ANP and cGMP plasma levels were measured in the range of 5-109 pg/ml (mean 45.5) and 0.2-2.8 pmol/ml (mean 1.3) respectively. In children with cardiac diseases significantly higher ANP (range 30-980, mean 280 pg/ml) and cGMP levels (range 0.2-6.0, mean 2.8 pmol/ml) were determined. There was a highly significant correlation between the two in children with cardiac diseases ($p < 0.001$). Furthermore, a significant correlation was found between right atrial pressure and ANP levels ($p < 0.01$) suggesting that atrial stretch is a stimulus for ANP release. The positive correlation between ANP and cGMP levels disagrees with the hypothesis that the cellular response to ANP is diminished in children with cardiac diseases.

37

W.Rascher*, T.Tulassay*, U.Heinrich, R.E. Lang* (Introd. by U.Heinrich)
Department of Pediatrics, University of Heidelberg, Fed. Rep. Germany
REGULATION OF PLASMA ATRIAL NATRIURETIC PEPTIDE IN CHILDHOOD

Atrial natriuretic peptide (ANP) is released from cardiac atria into circulation and induces diuresis and natriuresis. We studied plasma ANP in children using a sensitive radioimmunoassay. In 80 normal children aged 4 weeks to 16 years ANP averaged 27.8 ± 14.0 fmol/ml ($\bar{x} \pm SD$). Neonates had only slightly higher mean ANP levels, whereas in premature infants at day 1 of life ANP was up to 10 times higher than in normal children (252.6 ± 210.0 fmol/ml, $n:11$). The increase in plasma volume induced by albumin infusion in children with nephrotic syndrome ($n:9$) caused a rise in plasma ANP from 36.1 ± 22.6 to 151.4 ± 52.0 fmol/ml. ANP correlated with urine flow ($r:0.64$, $p < 0.01$) and with sodium excretion ($r:0.62$, $p < 0.01$). Reduction of volume overload by hemodialysis in children with chronic renal failure ($n:14$) resulted in a fall of elevated plasma ANP from 87.1 ± 39.4 to 42.8 ± 26.0 fmol/ml ($p < 0.001$). This decrease correlated with concomitant reduction of body weight. These observations suggest that volume expansion is a potent stimulus of ANP release in children and that this hormone is important in volume homeostasis.

Supported by Deutsche Forschungsgemeinschaft Ra 326/1-2

38

F.Peillon*, C.Liapi*, P.Garnier, A.M.Brandi*, D.Evain Brion, D.Bression*, H.Scherrer*, M.Donnadieu*
INSERM U 223, INSERM U 188, FRH, Paris, France

IN VITRO SIMULTANEOUS RELEASE OF ENDOGENOUS SOMATOSTATIN AND GROWTH HORMONE FROM HUMAN ADENOMATOUS PITUITARY CELLS

Dispersed cells obtained from 10 GH-secreting adenomas were studied in perfusion columns, eluted with Krebs Ringer buffer. Fractions were collected each 5 min for 5 hours and assayed by RIA for GH and SRIF contents. Basal GH secretion was variable (10 to 94 ng/ml/ 10^6 cells). Within the first 15 minutes of the perfusion SRIF was detected in 5 cases (5 to 41 pg/ml/ 10^6 cells). However in 5 cases a great release of SRIF was only observed after 90 minutes or more (7 to 25 pg/ml/ 10^6 cells). In 2 cases, during the perfusion, the variations of GH and SRIF levels were significantly negatively correlated ($p < 0.001$). Conclusions: 1) SRIF is released in vitro from human pituitary GH-secreting cells, 2) origin of SRIF release: internalization or synthesis? 3) the inverse correlation between SRIF and GH levels suggests a role of the intracellular SRIF in the regulation of GH secretion.

39

H.A. Delemarre-van de Waal*, E.J.M. Veldkamp* (Introd. by J.L. Van den Brande)
Department of Pediatrics, Academic Hospital of the Vrije Universiteit, Amsterdam, the Netherlands
LONG TERM TREATMENT WITH A SOMATOSTATIN-ANALOGUE (SMS 201-995) IN A PATIENT WITH PERSISTENT HYPOGLYCAEMIA DUE TO NESIDIIOBLASTOSIS

A Moroccan newborn female was admitted to the hospital directly after birth with asphyxia and convulsions. Laboratory examination revealed hypoglycaemia with elevated insulin levels. Glucose infusions in combination with diazoxide treatment could not maintain normoglycaemia. At 4 weeks of age subtotal pancreatectomy was performed. Pathological examination of the specimen showed nesidioblastosis. At 8.5 months, after a period of normoglycaemia, there was a reoccurrence of hypoglycaemia. Frequent feedings, diazoxide or glucagon treatment failed. At age 11 months SMS 201-995 was started. The dose was gradually increased from 20 once to 25 μ g 8 times per day (sc administration by a portable infusion pump, Zyklomat, Ferring, Kiel). Glucose levels were in the normal range, insulin levels declined. The patient was treated with SMS 201-995 for 17 months. After 9 months of treatment the dose could be decreased to 25 μ g 6 times per day sc. Height and weight gain are normal. During treatment normal growth hormone levels are found during sleep. Psychomotor development is appropriate for age. Short term interruptions of treatment do not elicit a rebound phenomenon with hypoglycaemia, in contrast to SMS 201-995 withdrawal for several hours. In conclusion: the long-acting somatostatin analogue (SMS 201-995) may be an alternative therapeutic approach in the long term management of hyperinsulinism.

40

P.Czernichow and C.Hellerström*
Hôpital des Enfants-Malades INSERM U30, Paris, France and Cell Biology, Uppsala, Sweden*.
INSULIN-LIKE GROWTH FACTOR I (IGF-I) IN THE RAT PANCREAS AND IN FETAL ISLETS IN CULTURE.

Growth hormone (GH) is a potent stimulator of the multiplication of pancreatic islet cells. Since in several tissues growth promoting effect of GH seems to be mediated through IGF production, we have attempted to characterize IGF-I and binding protein in total rat pancreatic extracts and in neonatal rat islet cells in culture. IGF-I was measured in acidic extract after gel filtration (biogel P100). RIA used specific antibody (NIAMDD), purified IGF-I (standard Dr Humbel) and 125 I-IGF-I (Dr Van den Brande). IGF-I is present in the pancreas of 18-21d fetus, neonatal and adult rats ranging from .24 to .07 ng/mg protein. A heavy molecular weight protein was characterized. At neutral pH 125 I-IGF-I was bound to this material and the tracer displaced by cold IGF-I after 4 hr incubation. IGF-I was also characterized in fetal rat islets in culture maintained for 3 days in serum free medium. It was found in the islets and in the medium together with a heavy molecular weight material with binding properties. Addition of GH in the medium (10 ng/ml) for 3 days increased the concentration of both IGF-I and binding protein in the medium.

In conclusion: IGF-I is present in the rat pancreas and in the medium of islets cells in culture together with a binding protein. Effect of GH on IGF-I production in the medium seems to indicate that GH multiplicative activity on β -cell might be mediated through local IGF-I stimulation.