51 REGULATION OF SOMATOMEDIN-BINDNING PROTEIN

Guilherme Pôvoa and Kerstin E Hall
Department of Endocrinology, Karolinska Hospital, Stockholm, Sweden

The low molecular form of somatomedin-binding protein (SMBP) in serum crossreacts in a radioimmunoassay developed for the SMBP isolated from human amniotic fluid. The SMBP in medium conditioned by hepatoma cells, HEP G2, was isolated by use of immunoaffinity chromatography and shown to have a N-terminal amino acid sequence identical with the amniotic SMBP. The HEP G2 cells were used as a model for the regulation of SMBP and the result was compared with those in humans.

The levels of immunoreactive SMBP are tenfold higher in newborns than in adults, in which they are inverse related to GH. Increased levels are found during pregnancy and in patients with uremia. Estrogens increase the levels whereas GH causes a suppression.

52 EXPERIMENTAL MODEL FOR DEGRADATION OF SOMATOMEDINS

Gösta Enberg & Arne Holmgren Depts of Endocrinology and Chemistry I, Karolinska inst.,Stockholm,Sweden.

The two somatomedins insulin-like growth factor I and II (IGF-I and II) purified from human plasma, have a high degree of sequence homology to proinsulin. Based on the known 3-dimensional structure of insulin this sequence homology has been used to predict conformational models of IGF-I and II. We therefore assumed that the insulin disulfide reducing thioredoxin system could be used as a model to study a possible way of degradation of IGF-I and II.

The experiments were performed by incubating 0.4-0.8 µM of each peptide together with thioredoxin and thioredoxinreductase in the presence of NADPH at room temperature. The reaction was terminated by addition of excess iodoacetic acid. The non-degraded material remaining after incubation, was measured with human placenta radioreceptor assay, with either insulin, IGF-I or II as labelled ligand.

The results show that both IGF-I and II were degraded by the thioredoxin system, with the same potency as insulin, which is in accordance with the predicted conformational models.

53 EFFECT OF A MONOCLONAL ANTIBODY AGAINST THE SOMATOMEDIN/INSULIN-LIKE GROWTH FACTOR TYPE I RECEPTOR ON IGF-II BINDING TO HUMAN PLACENTAL MEMBRANES.

Samuel J. Casella, Victor K.M. Han, A. Joseph D'Ercole, Marjorie E. Svoboda, Judson J. Van Wyk, The University of North Carolina School of Medicine, Department of Pediatrics, Chapel Hill, U.S.A.

Human placental membranes (HPM) have been employed in radioreceptor assays (RRA) for somatomedins/insulin-like growth factors (Sm/IGF). Although HPM contain both Sm/IGF Type I and II receptors, previous studies suggest that RRA's using HPM and either ^{125}I-Sm-C/IGF-I or ^{125}I-IGF-II primarily measure interaction with the Type I receptor (Nissley SP, Rechler MM, Clin Endo Metab, 13:43, 1984). We have found that a monoclonal antibody (αIR-3) to the Type I receptor which blocks ^{125}I-Sm-C binding does not block ^{125}I-IGF-II binding to HPM in the RRA. This suggests that the interaction of IGF-II with the Type I receptor is less than predicted by earlier competition studies. To further investigate the nature of IGF-II binding, ^{125}I-IGF-II was covalently crosslinked to HPM, solubilized, reduced and analyzed on SDS-PAGE. Autoradiography revealed a heavily labeled band at Mr 260K (Type II receptor), and a less intense band at Mr 135K. The Mr 135K band migrates nearly identically with the α subunits of both the Type I and insulin receptors; its intensity, however, was not attenuated by αIR-3 or by the presence of 100 ng/ml insulin. Parallel studies with radiolabeled Sm-C showed a marked inhibition of binding to the α subunit in the presence of αIR-3. These results suggest that IGF-II may bind to a 135K moiety that is discrete from previously described receptors. The nature of this moiety has not yet been defined.

54 STRUCTURAL SIMILARITIES AND DIFFERENCES AMONG THE IGF BINDING PROTEINS PRESENT IN THE TWO MACROMOLECULAR FORMS OF SERUM [IGF-BP] COMPLEXES.

S. Hardouin, P. Hossenlopp, L. Van Gennip and M. Binoux
INSERM U 142, Hôpital Trousseau, 75012 Paris, France.

Insulin-like growth factors (IGFs) are known to be carried in the blood by specific binding proteins (BPs) with which they form two complexes of apparent mol. wt. > 100 K ("big" complex) and 40-60 K ("little" complex). The former appears to be GH-dependent and consists of at least two subunits, one acid-stable which binds IGFs and the other acid-labile. To characterize the BPs after separation of the two complexes by gel filtration at pH 7.4, our approach has been to combine electroblotting with SDS-PAGE. With this technique the BPs are dissociated from their complexes and the free forms, tranferred on a nylon membrane, are detected by autoradiography after incubation with [^{125}I] IGF I. In the normal serum, two main bands (~ 38 and 42 K) were found both in the big and the little complex. Three extra fainter bands (~ 23, 28 and 34 K) were found in the latter. Similar pattern was obtained with hypopituitary serum although the proportion of the faster forms (~ 28 and 34 K) seemed to be increased. Binding material of mol. wt. > 100 K was also found in the big complex, only in the normal serum. All the bands were specific as shown with an excess of cold IGF. Conclusion : 1) The IGF BPs appear to be heterogenous. 2) Two main forms are found identical in the two complexes 3) The ratio of the various forms is different in the normal and the hypopituitary serum.

55

D H SINN*, A GROSSMAN*, A E JONES*, I DONLACH*, C CONNOLLY*, LESLEY H REES*, G M BESSER*, M O SAVAGE. Departments of Child Health, Endocrinology, Neurosurgery and Radiotherapy, St Bartholomew's Hospital, London EC1.

Prolactinoma in a prepubertal child: response to surgery, radiotherapy and dopamine agonist therapy.

An 8 year old boy presented with left proptosis, oculomotor weakness and bilateral galactorrhoea after a 4-year history of headaches. At craniotomy a large, infiltrating pituitary tumour was partially removed. At secondary referral GE9800 scan showed infiltration of the left cavernous sinus and orbit. Serum prolactin (Prl) ranged between 200,000 and 300,000 mU/l (normal <360 mU/l) and was unchanged on bromocriptine 30 mg/day which was poorly tolerated. Further tumour was excised at a second craniotomy. Histology showed an adenoma with frequent mitoses and positive immunostaining with Prl-antiserum in most cells. EM showed prominent rough endoplasmic reticulum but sparse neurosecretory granules and some microvilli. Immunostaining with colloidal gold Prl-antiserum demonstrated Prl within the granules Post-operatively Prl was 260,000 mU/l. Radiotherapy was given in a dose of 4500 cGy with the dopamine agonist mesulergine 10 mg daily. After one month, Prl fell to 83,500 mU/l. Mesulergine was replaced by bromocriptine 40 mg daily which, now well tolerated, induced a further fall in Prl to 40,200 mU/l where it has remained static. 9 months post-radiotherapy he is well with resolution of proptosis and oculomotor weakness. Tumour shrinkage was thus demonstrated with radiotherapy and dopamine agonist therapy in this tumour rarely seen in childhood.

56

M O SAVAGE, A GROSSMAN*, A BLACKLAY*, P S W DAVIES*, P N PLOWMAN*, D H COY*, G M BESSER. Departments of Endocrinology, Child Health, Paediatric Oncology and Radiotherapy, St Bartholomew's Hospital, London EC1A 7BE.

Cranial irradiation for cerebral tumours in childhood - evidence for a hypothalamic defect in growth hormone release.

Cranial irradiation, in a dose of 4500-5500 cGy was given to 9 patients (5 female) at ages ranging from 2-10 years for medulloblastoma (cranio-spinal irradiation: N=6), astrocytoma (N=1), optic glioma (N=1) and nasopharyngeal rhabdomyosarcoma (N=1). GH secretion was studied 29-126 months post-radiotherapy because of subnormal height velocity. Mean chronological age was 12.5 years and bone age 13.3 years. Mean height was -1.99 SDS compared with -0.60 SDS at the time of treatment. The patients who received spinal irradiation had evidence of diminished spinal growth; mean sitting height -3.60 SDS compared with subischial leg length -1.68 SDS. All subjects had impaired serum GH responses to insulin-induced hypoglycaemia (ITT); mean peak GH 4.5 mU/l (range 1-10.7 mU/l). The peak GH response to i.v bolus 200 µg GHRH(1-29)NH_2 was within the normal young adult range in 5 and marginally subnormal in 2, being in each case greater than during ITT; mean 22.8 mU/l (range 3.6-61.0 mU/l).

It is concluded that post-irradiation GH deficiency is (i) a common complication in patients treated for cerebral tumours in childhood, (ii) due to a functional disconnection between the hypothalamus and pituitary resulting in defective delivery of GHRH to the somatotrophs, (iii) potentially treatable with long-term synthetic GHRH.