Somatomedin levels in cerebrospinal fluid from adults with pituitary disorders

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Abstract. Somatomedin levels in cerebrospinal fluid (CSF) were determined in patients with acromegaly, pituitary deficiency, prolactinoma, and Cushing's disease by radioimmunoassay (RIA) for insulin-like growth factor 1 (IGF-1) and for IGF-2 as well as a radioreceptor assay (RRA) with adult human brain plasma membranes and IGF-2 as ligand. The mean value of RIA-IGF-2 (31 ± 1.6 ng/ml) predominated over that of RIA-IGF-1 (5.8 ± 0.3 ng/ml), but 10 times higher levels were found by RRA-IGF-2. Patients with acromegaly were not found to have higher values than those with GH deficiency even after corrections were made for possible leakage across the blood-CSF barrier. No correlations were found between CSF somatomedin levels determined by different techniques and immunoreactive IGF-1 or GH in the peripheral circulation except for a positive correlation between CSF RIA-IGF-2 and serum IGF-1 in patients with acromegaly. These findings suggest that somatomedins in CSF consist primarily of IGF-2-like peptides which are derived from production within the central nervous system or pituitary gland rather than from transport across the blood-CSF barrier.

Pituitary growth hormone (GH) is a prerequisite for normal growth and protein anabolism post-natally. The growth-promoting effect of GH is considered to be mediated by secondary polypeptide hormones belonging to the somatomedin family (Daughaday et al. 1972). In man, the purified somatomedins have been termed somatomedins A (SMA), somatomedin C (SMC), and insulin-like growth factors 1 and 2 (IGF-1 and IGF-2) (Rinderknecht & Humbel 1978a,b; Hall & Sara 1983). Recently SMC has been proven identical with IGF-1 and the only difference found between SMA and IGF-1 is glutamic acid instead of glutamine at position 40 (Klapper et al. 1983; Enberg et al. 1984). The IGF-1 peptide is GH regulated (Hall & Sara 1983). Patients with acromegaly and increased GH production have increased serum levels of IGF-1 related somatomedins, but normal levels of IGF-2 (Zapf et al. 1981; Enberg & Hall, in press). The factors regulating IGF-2 levels in the circulation are as yet not clarified. The liver is generally accepted as a production site for the somatomedins found in the circulation. Recent studies, however, have disclosed production of somatomedins in a variety of tissues including nervous tissue (d’Ercole et al. 1980; Binoux et al. 1981; Sara et al. 1982a, 1983).

The aim of the present study was to determine the somatomedin levels in cerebrospinal fluid (CSF) and to investigate if these levels show any relationship to GH or somatomedin in the peripheral circulation. The relation between somatomedin levels in CSF and serum was studied in patients with acromegaly, pituitary deficiency, prolactinoma, and Cushing's disease. Somatomedins were determined by radioimmunoassays for IGF-1 and IGF-2 and, in addition, a radioreceptor assay utilizing human brain membranes as matrix and IGF-2 as ligand.
Materials and Methods

Patients

Samples from serum and cerebrospinal fluid were obtained from patients subjected to encephalography during their stay at an in-patient ward at the Department of Endocrinology, Karolinska Hospital, Stockholm. Indication for the encephalography was evaluation of the tumour extension in the sella and suprasellar regions before operation or radiotherapy of the suspected adenoma. Exclusion criteria in this study were age above 65 years or diabetes mellitus requiring treatment other than diet. Patients with endocrine deficiencies were on substitution therapy. Patients were routinely given 15 mg of diazepam po and 0.5 mg atropine sc 1 h before lumbar puncture.

Patient groups consisted of 10 patients with active acromegaly, 9 patients with pituitary insufficiency ranging from isolated GH deficiency to panhypopituitarism, 12 patients with prolactinoma, and 8 patients with Cushing's disease. Some clinical and laboratory data from the patients are listed in Table 1.

Amongst the 10 patients with acromegaly, 6 had previously been operated upon. Post-operatively 1 patient had received stereotactic irradiation and one linear irradiation of the sella region. All patients had macroadeno¬mas, 3 with suprasellar and 1 with parasellar tumour extension. Five patients had no endocrine insufficiencies. Two had isolated hypogonadism, 1 hypogonadism together with hypothyroidism, and 2 insufficiencies of thyroid, gonadal, and ACTH-cortisol axis.

Allocated to the group with pituitary deficiencies were 1 patient with cranioopharyngeoma, 1 with hypophysitis, and 7 with non-endocrine chromophobic adenomas.

Four patients had previously been subjected to pituitary surgery. Suprasellar tumour extension was shown in 4 patients and small cisternal herniations were detected in 2 patients. All patients were GH deficient as judged from isolated GH levels and provocation tests. Two had isolated GH deficiency, in addition 2 patients had hypogonadism, 1 hypogonadism and hypothyroidism, and 4 panhypopituitarism. Five of the patients had slightly increased levels of prolactin.

In the group of 12 patients with prolactin-producing adenomas, 2 subjects had undergone pituitary surgery. Three of the patients had suprasellar tumour extension, 1 also with a large parasellar and frontal extension and 2 patients had tumour extension into the sinus cavities. Two patients were proved to have no hormone deficiencies, 2 were judged to have isolated deficiencies of GH, and 1 isolated gonadotrophin deficiency. The remaining 7 patients were considered to have deficiencies of both GH and gonadotrophins. Six patients had previously or recently been treated with bromoergocriptine and 1 was still on this treatment.

In the group of 8 patients with Cushing's disease 1 patient had previously been subjected to hypophysal surgery and stereotactic irradiation. Two patients had small suprasellar tumour extension. Urinary cortisol excretion ranged from 230–2125 nmol/24 h (normal range 80–300 nmol/24 h). Two patients were suspected to have isolated GH deficiency but the remaining patients had no endocrine insufficiencies.

Samples

Blood samples were obtained from fasting, resting subjects in the morning before the radiological examination

<table>
<thead>
<tr>
<th>Table 1.</th>
<th>Some clinical and laboratory data in four groups of adults with pituitary disorders.</th>
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</thead>
<tbody>
<tr>
<td>Number</td>
<td>Acromegaly</td>
</tr>
<tr>
<td>Sex, females : males</td>
<td>7 : 3</td>
</tr>
<tr>
<td>Age, years</td>
<td>36–62</td>
</tr>
<tr>
<td>Height, cm</td>
<td>173 ± 14</td>
</tr>
<tr>
<td>Serum GH, µg/l</td>
<td>8–270</td>
</tr>
<tr>
<td>Serum prolactin, µg/l</td>
<td>2–24</td>
</tr>
<tr>
<td>Fasting blood glucose, mmol/l</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>CSF glucose, mmol/l</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Serum albumin, g/l</td>
<td>44.0 ± 6.0</td>
</tr>
<tr>
<td>CSF albumin, mg/l</td>
<td>311 ± 115</td>
</tr>
<tr>
<td>Albumin ratio x 100 (CSF:serum)</td>
<td>0.61 ± 0.28</td>
</tr>
</tbody>
</table>

CSF indicates cerebrospinal fluid. Numerical data are expressed as mean ± sd or range.
and serum was frozen within 2 h. Lumbar cerebrospinal fluid was collected in 2 consecutive portions of approximately 2 ml. The cerebrospinal fluid was immediately transferred to an ice bath, centrifuged, and frozen within 2 h. From 10 patients with acromegaly both CSF portions were analysed for RIA-IGF-1, otherwise all determinations were performed in the second portion of CSF. Acid ethanol extraction of the samples was performed as described by Goldberg et al. (1982).

**Assays**

Immunoreactive IGF-1 in serum and CSF was determined by the radioimmunoassay for SMA as described previously (Hall et al. 1979). Pure IGF-1, isolated according to Enberg et al. (1984) was used as ligand and standard. The preparation used for immunization was not completely pure but contaminated with IGF-2. In this assay the cross-reaction of IGF-2 is 1% and insulin does not cross-react.

Immunoreactive IGF-2 was determined in CSF directly or following acid ethanol extraction (AE) of CSF. The RIA-IGF-2 assay is a modification of a newly developed RIA for IGF-2 (Enberg & Hall, in press). After an incubation period of 36 h at 4°C the unbound IGF-2 was adsorbed to Neutral Charcoal Sigma 5385 before separation. The cross-reaction with IGF-1 was 8–10%. The values are expressed in ng equivalents of pure IGF-2, kindly provided by R. Humbel, Zürich, Switzerland. The

arbitrary reference serum was defined as containing 1 U/ml, equipotent with 180 ng IGF-1 per ml or 1000 ng IGF-2 per ml.

Somatomedins in CSF were also analysed in a radio-receptor assay with IGF-2 as ligand and adult human brain membranes as matrix (Sara et al. 1982b). In this assay IGF-1 and IGF-2 related peptides are equipotent. The arbitrary reference serum was equipotent with 1000 ng IGF-2 per ml.

Growth hormone was assayed in serum by a double antibody radioimmunoassay modified after Cerasi et al. (1966), normal value < 9 µg/l. Prolactin values were determined using a commercial radioimmunoassay kit (Prolactin kit, Serono Diagnostics), normal range in serum 3–19 µg/l. Albumin in serum and CSF was determined by a turbidimetric method (Lizana & Hellsing 1974), normal ranges 37–52 g/l and 70–400 mg/l, respectively; albumin ratio (CSF: serum) × 100, normal range 0.20–0.90. Glucose in blood and CSF was determined by a glucose oxidase method (Hugget & Nixon 1957), normal range for blood glucose 3.0–6.0 mmol/l.

**Statistics**

In the text mean values are given together with SEM. Statistical analyses were performed using non-parametric methods. Two-sided Mann-Whitney and Spearman-Rank tests were used for comparison between groups and correlations between parameters. Differences be-
between results in consecutive samples of CSF and differences in results between direct IGF-2 assays and assays following AE were analysed with Wilcoxon matched-pairs signed ranks test. In all cases $P \leq 0.05$ was chosen as the level of statistical significance.

Results

In the RIA-SMA the displacement curves of both serum and CSF were superimposable on that for the pure IGF-1. In the RIA-IGF-2 using charcoal separation the dose response curve of CSF, but not serum, was parallel to that of the IGF-2 peptide (Fig. 1). Acid extracted (AE) CSF was slightly more potent in displacing the labelled IGF-2. Although the AE technique did not allow determination at high concentrations with confirmation of parallelity, values determined both directly and after AE extraction are presented. There was no significant difference in immunoreactive IGF-1 values between the first and second CSF portions.

The mean CSF levels of somatomedins determined by 3 different assays are shown in Table 2. No age-dependency was observed, but a positive correlation was found between height and the CSF levels of immunoreactive IGF-2 ($P < 0.01$). In the combined material the mean level of RIA-IGF-1 in CSF was low (5.8 ± 0.3 ng/ml), reaching only 3% of the corresponding serum level. No correlation was found between the individual levels of RIA-IGF-1 in CSF and serum as seen from Fig. 2. Neither was any relation found between RIA-IGF-1 in CSF and GH in serum. The lowest CSF level of immunoreactive IGF-1 was found in patients with prolactinoma (4.0 ± 0.6 ng/ml), but this value was significantly different ($P < 0.02$) from the levels observed in patients with GH deficiency or Cushing’s disease only. In the combined material, a negative correlation was observed for the serum concentration of prolactin ($r = -0.34, P < 0.05$). This was due to the contribution of the prolactinoma group.

The concentration of immunoreactive IGF-2 in CSF was 5–6-fold higher than that of immunoreactive IGF-1. The mean level determined in AE extracted CSF (44 ± 1.7 ng/ml) was higher ($P < 0.001$) than that performed directly in CSF (31 ± 1.6 ng/ml). Prolactinoma patients showed the lowest content of RIA-IGF-2 when determinations were performed directly in CSF. The mean level was significantly ($P < 0.02$) lower compared with the patients with Cushing’s disease. This difference disappeared when IGF-2 was determined in AE extracted CSF. A positive correlation was found between immunoreactive IGF-2 and immunoreactive IGF-1 when assayed directly in CSF ($r = 0.44, P < 0.01$). Apart from a correlation between the CSF RIA-IGF-2 and serum RIA-IGF-1 within the acromegaly group, no correlation was found between CSF RIA-IGF-2 and GH or RIA-IGF-1 in the circulation. Similarly to the CSF RIA-IGF-1 values, the RIA-IGF-2 showed a negative correlation to serum prolactin levels ($P < 0.01$), which disappeared in AE extracted CSF. Somatomedins determined by brain RRA-IGF-2, in which IGF-1 and IGF-2 are equipotent resulted in CSF levels.

Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Acromegaly ng/ml</th>
<th>Pituitary deficiency ng/ml</th>
<th>Prolactinoma ng/ml</th>
<th>Cushing’s disease ng/ml</th>
<th>Combined groups ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 10)</td>
<td>(n = 9)</td>
<td>(n = 12)</td>
<td>(n = 8)</td>
<td>(n = 59)</td>
</tr>
<tr>
<td>S-RIA IGF-1</td>
<td>505.8 ± 315.0</td>
<td>84.6 ± 28.8</td>
<td>86.4 ± 30.6</td>
<td>144.0 ± 46.8</td>
<td></td>
</tr>
<tr>
<td>CSF-RIA IGF-1</td>
<td>5.9 ± 1.6</td>
<td>7.0 ± 1.6</td>
<td>4.0 ± 1.8</td>
<td>7.0 ± 1.6</td>
<td>5.8 ± 2.2</td>
</tr>
<tr>
<td>CSF-RIA IGF-2</td>
<td>31 ± 8</td>
<td>33 ± 8*</td>
<td>25 ± 7</td>
<td>39 ± 13</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>CSF-AE-RIA IGF-2</td>
<td>43 ± 12</td>
<td>43 ± 7*</td>
<td>43 ± 8</td>
<td>47 ± 14</td>
<td>44 ± 10</td>
</tr>
<tr>
<td>CSF-RRA IGF-2</td>
<td>265 ± 83</td>
<td>325 ± 99</td>
<td>230 ± 83**</td>
<td>465 ± 82</td>
<td>315 ± 122</td>
</tr>
</tbody>
</table>

n indicates total number of patients in each group; * n = 8 and ** n = 11. AE indicates acid ethanol extraction.

RRA IGF-2 values are expressed as ng equivalents/ml. Values are expressed as mean ± sd.
which were considerably higher than the combined amount of immunoreactive IGF-1 and IGF-2. The level of RRA-IGF-2 correlated positively to immunoreactive IGF-2 \((r = 0.44, P < 0.01)\) and immunoreactive IGF-1 \((r = 0.36, P < 0.05)\).

The present results may have been influenced by blood-CSF barrier defects in the various patient groups. Only 4 of the patients (2 with acromegaly, 1 with prolactinoma, and 1 with Cushing's disease) showed a pathologically elevated CSF:serum albumin ratio. In spite of this, a positive correlation between the immunoreactive IGF-1 and IGF-2 in CSF and the albumin ratio (CSF:serum) was observed \((P < 0.05\) and \(P < 0.01\), respectively).

To evaluate the possible influence of leakage across the blood-CSF barrier each individual somatomedin value in CSF was expressed in relation to the albumin ratio (CSF:serum) as shown in Fig. 3. Patients with acromegaly displayed significantly lower values as compared to patients with pituitary deficiency \((P < 0.02—0.05)\). Decreased values in acromegaly were also observed in comparison with Cushing’s disease when CSF RRA-IGF-2:albumin ratio was used. In addition, the patients with pituitary deficiency showed higher CSF RIA-IGF-1:albumin ratio as compared to patients with prolactinoma or Cushing’s disease. Utilizing the CSF albumin concentrations for correction of leakage instead of the albumin ratio (CSF:serum) resulted similarly in higher values for the patients with pituitary deficiency as compared to acromegaly \((P < 0.05)\). These findings clearly demonstrate that CSF somatomedin concentrations are independent of GH production as reflected by the GH and RIA-IGF-1 in the periphery.

**Discussion**

The present study confirms the previous finding of immunoreactive IGF-1 and IGF-2 in human CSF by Haselbacher & Humbel (1982). In accordance with these authors immunoreactive IGF-2 in CSF was found to be 5—10-fold higher than immunoreactive IGF-1 although both levels are approximately 20-fold lower than in the serum of healthy adults. The mean level of immunoreactive IGF-2 in AE extracted CSF found in the present study is comparable to the level reported in CSF for total IGF-2 content after separation by gel chromatography at low pH (Haselbacher & Humbel 1982). These authors demonstrated equal amounts of two molecular forms of IGF-2, a ‘big’ IGF-2 with a
Cerebrospinal (CSF) somatomedins expressed as a ratio between determined levels and the albumin ratio (CSF: serum) in patients with acromegaly, pituitary deficiency, prolactinoma, and Cushing's disease. The following assays were used: radioimmunoassay (RIA) IGF-1, RIA IGF-2 performed directly in CSF (ORIG) or following acid ethanol extraction (AE), radioreceptor assay with human brain membranes and IGF-2 as ligand (RRA IGF-2). Mean values are indicated as horizontal lines. Statistically significant differences between patient groups are indicated by horizontal arrows (* $P \leq 0.05$ and ** $P \leq 0.01$).

Molecular weight of approximately 9000 besides the regular form of IGF-2. In our study these two forms have not been separated. The 'big' IGF-2 was reported to be more potent in a bioassay than in radioimmunoassay in comparison with IGF-2. This may partly explain our finding of much higher values of somatomedins in CSF when determined in the RRA than in the RIA. Beaton et al. (1975) have earlier reported comparable levels of somatomedin activity in CSF when using the por-
cine cartilage bioassay. Although the higher RRA level may be partly due to interference with carrier proteins this cannot explain the enhanced bioactivity.

There was no age-dependency but surprisingly a positive correlation was found between height and immunoreactive IGF-2 opposite to the negative correlation observed between height and amine metabolites in lumbar CSF (Wode-Helgødt & Sedvall 1978).

In contrast to the GH dependency of somatomedin in the circulation neither immunoreactive IGF-1 nor IGF-2 was higher in patients with acromegaly compared to those with GH deficiency. Apart from the positive correlation between CSF RIA-IGF-2 and serum RIA-IGF-1 within the acromegaly group, no relation was found between somatomedin in CSF and somatomedin or GH levels in the peripheral circulation. A lack of correlation was observed both before and after correction for possible leakage over the blood-CSF barrier as assessed by the albumin ratio (CSF:serum). These findings indicate the existence of a blood-CSF barrier for the somatomedins. The difference in CSF somatomedin levels between prolactinoma patients and those with Cushing's disease which decreased following correction for blood-CSF barrier leakage indicates the existence of a changed transport of somatomedins in these groups. Somatomedins present in the CSF are, probably mainly derived from central nervous system production. This is in accordance with earlier studies demonstrating endogenous production from brain tissue and pituitary gland in vitro (d'Ercole et al. 1980; Binoux et al. 1981; Sara et al. 1983).

Acknowledgments

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References


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