EFFECT OF INTRAVENOUS ADMINISTRATION OF HUMAN GROWTH HORMONE ON SULPHATION FACTOR ACTIVITY IN SERUM OF HYPOPITUITARY SUBJECTS

By

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ABSTRACT

Human growth hormone (HGH) administered as an iv injection of 2–4 mg to hypopituitary patients induced a rise in the levels of sulphation factor (SF) in serum. The low basal levels of SF were not changed during the first hour after HGH injection. Not until three hours after injection, when HGH values approached basal values, there was a significant rise in SF. The mean difference of SF at one and at three hours after HGH injection was 0.52 ± 0.11.

The presence in human serum of factors which stimulate the in vitro incorporation of labelled sulphate into cartilage is well established (Daughaday et al. 1959; Almqvist 1961; Yde 1968; Hall 1970). This sulphation factor (SF) activity in serum seems to be correlated in some way to growth hormone. Thus, low levels of SF activity were reported in pituitary dwarfism (Almqvist 1960; Fraser & Hartog 1962; Kogut et al. 1963; Daughaday & Parker 1963; Brasel et al. 1965; Daughaday & Kipnis 1966; Hall 1970), and they increased after im administration of human growth hormone (HGH) (Almqvist 1960; Parker et al. 1964; Daughaday & Kipnis 1966). In acromegaly, the SF levels were mostly increased (Almqvist et al. 1961; Fraser & Hartog 1962; Hall 1970).

Since HGH in vitro has no stimulatory effect on the sulphate incorporation into cartilage (Salmon & Daughaday 1957; Hall 1970), it has been accepted that the hormone induces the appearance in serum of SF activity. One way of elucidating the connection between HGH and SF would be to study the time relationship between the administration of HGH and the appearance of
SF activity. This was done in the present study in which SF activity was measured at different time intervals after a single iv injection of HGH.

**MATERIAL AND METHODS**

**Case material**

Eight patients with low levels of SF activity were investigated. One (B) was an adult woman with a hypothalamic tumour, previously treated with irradiation. She had diabetes insipidus, primary amenorrhoea and no rise in HGH in plasma during insulin induced hypoglycaemia. The 7 other patients showed growth retardation. Clinical and laboratory data in these subjects are given in Table 1. The 3 patients with panhypopituitarism (Nos. 5, 6 and 7) were given their usual replacement of cortisone and thyroxine during the investigation. Patient No. 4 had an isolated growth hormone deficiency but had menarche at 16 years of age. Nos. 4 and 5 had been treated with human growth hormone for 2 years; this treatment was withdrawn 1 month and 1 year, respectively, before the investigation. One of the patients, No. 7, had a craniopharyngeoma, and was operated upon at 4 years of age.

**Methods**

HGH and insulin were determined by radioimmunoassay, HGH according to Cerasi et al. (1966), and insulin according to Hales & Randle (1963). Glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi reagens). The SF activity was measured by a technique using embryonic cartilage as described in a previous paper (Hall 1970). In all instances a 4-point or 6-point assay was performed, and values not fulfilling the criteria of parallelism and linearity were rejected.

The HGH used in the present work was a gift from AB Kabi, Stockholm, prepared according to Roos et al. (1963). One mg of this HGH preparation corresponded to 2 U of the first International Reference Preparation of human growth hormone (National Institute for Medical Research, London) as determined by radioimmunoassay.

All patients were hospitalized during the study. Blood samples were taken after an overnight fast. They were given 2 or 4 mg (4 or 8 U) of human growth hormone in 5 ml of saline as an iv injection during 4–5 min. Venous serum samples were taken before and every 30 min up to 2 h and then at longer intervals.

**RESULTS**

In Fig. 1, the HGH and SF values in the adult patient with growth hormone deficiency are shown. One hour after the injection of 4 mg (8 U) of HGH, when the HGH levels in plasma were still high, there was no discernible effect on the SF levels. Not until 3 h after the HGH administration, when the injected HGH had nearly disappeared from plasma, did the SF activity increase and it remained elevated during the following 24 h.

In Fig. 2 and Table 2, the HGH, glucose and SF levels in the 7 pituitary dwarfs are shown. Two mg of HGH were given iv regardless of the patients' body weight. In patient No. 1, the injection with 2 mg (4 U) of HGH was
Table 1.
Clinical and laboratory data in 7 patients with pituitary dwarfism.

<table>
<thead>
<tr>
<th>Subject</th>
<th>1 J. S.</th>
<th>2 E. H.</th>
<th>3 L. W.</th>
<th>4 Y. F.</th>
<th>5 L. R.</th>
<th>6 B. M.</th>
<th>7 G. A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex and age</td>
<td>M 12.78</td>
<td>M 16.51</td>
<td>F 13.16</td>
<td>F 20.03</td>
<td>M 23.05</td>
<td>M 24.73</td>
<td>F 17.76</td>
</tr>
<tr>
<td>Height, cm</td>
<td>127.0</td>
<td>138.5</td>
<td>124.0</td>
<td>145.0</td>
<td>145.0</td>
<td>147.5</td>
<td>129.5</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>26.0</td>
<td>28.8</td>
<td>20.7</td>
<td>41.0</td>
<td>47.6</td>
<td>35.5</td>
<td>28.0</td>
</tr>
<tr>
<td>Bone age, year</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>18.5</td>
<td>14</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Growth rate preceding HGH treatment, cm/year</td>
<td>3.0</td>
<td>3.3</td>
<td>2.4</td>
<td>2.5</td>
<td>1.5</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>HGH increase during insulin tolerance test, ng/ml</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>SF, U/ml</td>
<td>0.53</td>
<td>0.48</td>
<td>0.29</td>
<td>0.38</td>
<td>0.20</td>
<td>0.38</td>
<td>0.42</td>
</tr>
<tr>
<td>Fiducial limits of error</td>
<td>(0.33–0.77)</td>
<td>(0.34–0.75)</td>
<td>(0.04–0.79)</td>
<td>(0.14–0.79)</td>
<td>(0.08–0.35)</td>
<td>(0.24–0.59)</td>
<td>(0.14–1.01)</td>
</tr>
</tbody>
</table>

P = 0.95

Treatment:
thyroxine, mg/day
0.20
0.20
None
None
None
0.20
0.20
0.20
cortisone, mg/day
None
None
None
None
15
15
25
Levels of human growth hormone (HGH) and sulphation factor (SF) activity in serum from a grown-up woman with hypopituitarism before and after iv injection of 4 mg (8 U) of HGH.

repeated after an interval of 6 months. The HGH levels at 30 and 60 min showed great variation, and the half-times of disappearance of HGH from plasma ranged from 18 to 35 min. Blood glucose in 6 of the 7 patients showed a slight decrease 30 min after HGH injection. The mean of the difference of glucose before and 30 min after HGH injections was 14 mg/100 ml.

The low basal values of SF were not changed during the first hour after HGH injections. Not until 3 h after the injection, when the HGH values approached normal values, there was a significant rise in SF activity. The mean difference of SF activity at 1 and 3 h after HGH injection was $0.52 \pm 0.11$ (n = 5). In patient No. 7, who had received 12.5 mg of cortisone before the HGH was given, there was no discernible effect on SF.

Insulin in plasma was determined in some blood samples and was decreased after HGH injection (Table 2).

As seen in Table 2, there was no correlation between SF values and corresponding levels of HGH, glucose, or insulin, but HGH induced a rise in SF values after 3 h.

**DISCUSSION**

The results of the present investigation show that HGH induces the appearance of a factor or factors in human serum, which are capable of stimulating sulphate incorporation into cartilage. This factor can not be growth hormone.
Fig. 2.
Levels of human growth hormone (HGH), glucose and sulphation factor (SF) activity in 7 pituitary dwarfs before and after iv injection of 2 mg (4 U) of HGH. Dotted lines represent patients, who received substitution therapy with cortisone.

Table 2.
HGH, glucose and SF values (mean ± sem) before and after iv injection of 2 mg of HGH to pituitary dwarfs.

<table>
<thead>
<tr>
<th></th>
<th>Basal values</th>
<th>60 min after HGH</th>
<th>180 min after HGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGH, ng/ml</td>
<td>5.9 ± 0.6</td>
<td>93.4 ± 12.7</td>
<td>14.9 ± 2.9</td>
</tr>
<tr>
<td></td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td>17.5 ± 1.2</td>
<td>11.7 ± 1.0</td>
<td>13.8 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>(n = 4)</td>
<td>(n = 6)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>Glucose, mg/100 ml</td>
<td>79 ± 6</td>
<td>72 ± 3</td>
<td>89 ± 7</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>SF, U/ml</td>
<td>0.35 ± 0.04</td>
<td>0.37 ± 0.05</td>
<td>0.82 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 5)</td>
<td>(n = 8)</td>
</tr>
</tbody>
</table>
itself since the activity did not appear until the radioimmunological levels of HGH had decreased. It can not be excluded that SF might be a degradation product of HGH. However, the fact that a high level of SF activity remained as long as 24 h after one single injection of HGH speaks against this possibility.

It is well known that amino acids influence the incorporation of labelled sulphate into cartilage (Salmon & Daughaday 1958; Salmon 1960; Adamson et al. 1966; Hall 1970). To exclude the possibility that amino acids could be the inducible factor, the biological assay was always performed in a medium containing maximal amounts of amino acids (Hall 1970). Furthermore, determinations of amino acids in plasma were performed in hypophysectomyed dwarfs before and after HGH injection, and a decrease of some amino acids was noted (Hall et al., to be published).

Insulin in high doses is known to stimulate the incorporation of sulphate into cartilage (Salmon & Daughaday 1957; Salmon 1960). However, this effect can not be obtained with insulin in physiological amounts. Furthermore, after iv injection of 2 mg of HGH there was a slight decrease in the levels of radioimmunological insulin levels, which is in accordance with earlier result in fasting subjects (Frohman et al. 1967).

In normal children (Daughaday & Kipnis 1966) and in serum from fasting normals and diabetic subjects (Yde 1969) no correlation was found between the values for HGH and SF. In normal subjects the HGH levels show diurnal variations, and increases in the HGH are induced by many stimuli. Since the half-time of disappearance of HGH in plasma is short (Refetoff & Sönksen 1970) and a period of 3 h elapses before the SF values increase as demonstrated here, the lack of correlation between HGH and SF values in normal individuals is not surprising.

It is of some interest in this connection that another biological activity, insulin-like activity (ILA), was shown to be increased about 4 h after iv injection of HGH (Zahnd et al. 1960). The possible relationship between SF and ILA is under study by the author.

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REFERENCES

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