The effect of glibenclamide on plasma insulin, plasma somatomedin bioactivity and skeletal growth in hypophysectomized rats

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Abstract. Male rats, body weight 60–75 g, were hypophysectomized. Three days after operation the animals were divided into two groups. Group B received solvent solution and group C 1 mg/kg body weight per day of glibenclamide ip for the following 9 days. Group A consisted on non-operated normal rats. Twenty-four hours after the last injections and after a 12 h overnight fast the body weights of groups B and C were not different, the increase during the 10 days being 10% in both groups. Serum insulin (IRI) was significantly higher in group C than in group B (C: 8.0 ± 0.3 μU/ml, n = 14 vs B: 4.9 ± 1.0 μU/ml, n = 14; P < 0.01, mean ± SEM) as was serum somatomedin bioactivity (SM)-porcine cartilage assay – (C: 1.06 ± 0.1 U/ml, n = 14 vs B: 0.41 ± 0.01 U/ml, n = 14; P < 0.001). Skeletal growth was determined with the tibia test and by a radiograph of each rat. The width of the proximal epiphyseal growth plate of the tibia was significantly increased in group C compared to group B (C: 204 ± 4.8 μm, n = 12 vs 181 ± 6.5 μm, n = 13; P < 0.005). On the radiograph the area of the right femur was not different between the two groups of animals, while the height and the area of the first lumbar spine were significantly augmented in group C. The results show that glibenclamide stimulates IRI, SM and skeletal growth in hypophysectomized rats. Compared to the glibenclamide treated hypophysectomized animals the normal rats of group A had doubled their body weights. IRI (59 ± 5 μU/ml, n = 4) and skeletal growth (tibia test: 454 ± 5.8 μm) were greatly increased. SM did not differ between group A (1.21 ± 0.35 U/ml) and group C. T₄ was much lower in group B (0.64 ± 0.09 μg/100 ml, n = 5) than in group A (4.1 ± 0.3 μg/100 ml, n = 6; P < 0.001).

It is concluded that a normal SM concentration is not necessarily associated with appropriate growth.

Salter & Best (1953) injected hypophysectomized rats with a long acting insulin preparation and demonstrated a definite augmentation of skeletal growth. This was the first experimental evidence that insulin may act as a growth hormone. At that time it was unknown whether insulin enhances skeletal growth directly or via the generation of growth factors. During the following years the somatomedins were identified. These growth hormone-dependent polypeptides, in contrast to growth hormone, stimulate cartilage metabolism in vitro and are thought to mediate the effect of growth hormone on the skeleton in vivo (Daughaday 1973; Daughaday et al. 1972; Van Wyk et al. 1974; Zapf et al. 1978; Hall & Fryklund 1979). More recently, it has been shown that perfusion of rat livers with insulin results in an increased somatomedin concentration in the perfusate suggesting a direct effect of insulin on the generation of the somatomedins from the livers. However, pharmacological doses of 1000 μU/ml insulin had to be used to liberate somatomedin (Daughaday et al. 1976; Shapiro et al. 1978). Therefore, in the present experiments the effect of endogenous
insulin, in a probably more physiological concentration, on serum somatomedin and some other growth parameters was studied in hypophysectomized rats.

Material and Methods

Forty-three male Wistar rats, weighing 60–75 g, were used. Ten rats were not operated and served as normal group A. The remaining rats were hypophysectomized under general anaesthesia (Nembutal®; 40 mg/100 g body weight) by a modified stereotactic transauricular method previously described (Baetzner et al. 1972).

Three days after operation the animals were randomly divided into two equal sized groups B and C. On the next 9 days the animals were weighed between 9 and 10 a.m. and injected ip with a volume of 0.02 ml per 10 g body weight. The normal group A and the hypophysectomized group B received solvent solution whereas the hypophysectomized group C was injected with 1 mg/kg glibenclamide (kindly provided by Professor F. Schmidt, Boehringer, Mannheim, Germany).

According to the manufacturer (Boehringer, Mannheim) on each day 50 mg glibenclamide was suspended in 1 ml ethanol and dissolved in 2 ml 0.1 M potassium chloride. Aqua bidist. was added as was 0.1 M NaH2PO4 to adjust for pH 9. The final glibenclamide concentration was 50 mg/100 ml.

The rats had free access to water and food (rat pellets Altromin®, Altrogge, Lippe, Germany). For 3 days after operation the food was soaked in water. Twenty-four hours after the last injection and after a 12 h overnight fast the animals were anaesthetized. Blood was collected from the abdominal aorta, centrifuged and the plasma stored at −20°C until analysis. After sacrifice, radiographs were taken from the rats in a straight dorsoventral position at a tube distance of 1 m. Subsequently, the right tibia was removed.

Insulin was measured radioimmunologically (Insik-1-kit®; Isotopen-Dienst-Werk, Dreieich, Germany) with rat insulin as standard (Lot No.: R 171; Novo, Copenhagen, Denmark) as was thyroxine (Serlaute®, Miles-Ames, Frankfurt/Main, FRG). Plasma somatomedin was determined with the porcine cartilage bioassay according to Van den Brande & Caju (1974). The pooled plasma of 20 healthy male volunteers was used as standard for the somatomedin bioactivity. By definition 1 ml of the standard plasma contains 1 U somatomedin. The tibia specimens were examined for skeletal growth. The mean width of the proximal epiphyseal cartilage zone was measured at 10 equidistant sites. Details are described by Baetzner et al. (1972).

The radiographs were examined for bone size. The height of the first lumbar vertebra was measured with a calibrated filar magnifying glass. Area measurements of the first lumbar vertebra and the right femur were made with a computerized image analyzing system (Kontron; Eching, Germany), where the perimeter of the area was traced with a special stylus.

The results of the somatomedin determinations were evaluated by standard statistical methods for bioassays (Finney 1952). The statistical significance of all the results between the groups was calculated using Student’s t-test.

Results

Body weight, thyroxine, plasma insulin, and plasma somatomedin

Treatment was initiated 3 days after hypophysectomy. At that time and at the end of the experiments 10 days later the body weights of the solvent

Table 1.

<table>
<thead>
<tr>
<th>Rats</th>
<th>n</th>
<th>Weight (g)</th>
<th>IRI (μU/ml)</th>
<th>SM (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
<td>Day 10</td>
<td>Day 10</td>
</tr>
<tr>
<td>Normals</td>
<td>4</td>
<td>86 ± 1.4</td>
<td>153 ± 2.4</td>
<td>59 ± 5</td>
</tr>
<tr>
<td>Hypophysectomized</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td>14</td>
<td>71 ± 1.5</td>
<td>79 ± 1.7</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>14</td>
<td>73 ± 1.2</td>
<td>81 ± 1.7</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>n. s.</td>
<td>n. s.</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Table 1.

Body weight, serum insulin (IRI), and serum somatomedin (SM) in normal and hypophysectomized rats. The hypophysectomized rats were injected for 9 days with solvent solution or with 1 mg/kg body weight per day glibenclamide. Mean ± SEM; P denotes the difference between solvent and glibenclamide. n.s. = not significant.
and glibenclamide treated groups B and C were statistically not different. The weight gain in the hypophysectomized groups B and C was approximately 1 g per day while that of the non-operated group A was 6.7 g per day (Table 1).

Serum thyroxine was measured in 6 non-operated group A and 5 hypophysectomized solvent treated group B rats. T₄ amounted in group A to 4.1 ± 0.3 μg/100 ml and in group B 0.64 ± 0.09 μg/100 ml. The difference was highly significant (P < 0.001).

The insulin determinations were performed after a 12 h overnight fast and 24 h after the last injection. Hypophysectomy resulted in a greatly diminished plasma insulin concentration irrespective of whether the animals were injected with solvent or glibenclamide. However, treatment of the hypophysectomized group C with the sulphonylurea increased the plasma insulin concentration significantly when compared with the solvent treated group B.

In the same plasma samples the somatomedin (SM) concentration was determined. In the solvent treated group B SM was significantly decreased, and in the glibenclamide treated group C SM was elevated towards normal when compared with the non-operated group A (Table 1).

Skeletal growth and bone size parameters
The morphological evaluation of the growing skeleton was performed by two methods:

<table>
<thead>
<tr>
<th>Bone</th>
<th>Treatment</th>
<th>Hypophysectomy</th>
<th>Normals</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solvent</td>
<td>n</td>
<td>Glibenclamide</td>
<td>n</td>
</tr>
<tr>
<td>Tibia test (μm)</td>
<td>181 ± 6.5</td>
<td>12</td>
<td>204 ± 4.8</td>
<td>13</td>
</tr>
<tr>
<td>Lumbar spine</td>
<td>Height (mm)</td>
<td>3.61 ± 0.06</td>
<td>13</td>
<td>3.77 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Area (mm²)</td>
<td>15.07 ± 0.21</td>
<td>13</td>
<td>17.30 ± 0.16</td>
</tr>
<tr>
<td>Femur</td>
<td>Area (mm²)</td>
<td>50.04 ± 0.39</td>
<td>13</td>
<td>47.64 ± 0.28</td>
</tr>
</tbody>
</table>

Table 2.
Skeletal growth in normal and hypophysectomized rats. The tibia test and a X-ray was done at the time of sacrifice on day 10. On the radiograph the height and the area of the first lumbar spine and the area of the right femur were evaluated. Mean ± SEM; P denotes the difference between solvent and glibenclamide. n.s. = not significant.

In the modified tibia assay microphotograms of sections of the proximal epiphyseal growth plate were evaluated. When compared with the non-operated group A, removal of the pituitary resulted in a diminished width of the growth plate both in the solvent and the glibenclamide treated groups B and C. When the hypophysectomized groups B and C were compared the width of the growth plate of the sulphonylurea treated group C was significantly increased.

By morphometric analysis of the radiographs, height and area of the first lumbar vertebra were significantly increased in the glibenclamide group C as compared with the solvent group B whereas the area of the right femur was statistically not different between groups B and C.

In the normal group A the three bone size parameters were much greater than in the hypophysectomized animals (Table 2).

Discussion

From clinical and experimental studies it is well established that growth hormone promotes the generation of somatomedins (SMs), a family of polypeptides that is thought to mediate at least some of the actions of growth hormone on its target tissues in vivo (Daughaday 1973). In growth hormone deficient dwarfs serum SM concentrations are low and treatment of the patients with

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somatotrophin restores the decreased somatomedin towards normal (Hizuka et al. 1978; Hall et al. 1980). Experimentally, hypophysectomy of normal rats is associated with a decrease of the SM levels within 2 days of operation and treatment of the animals with growth hormone increases the low levels of the growth factor to pre-operative concentrations (Takano et al. 1978; Kaufmann et al. 1978). In the opposite endocrinological situation such as in patients with active acromegaly, high concentrations of growth hormone and elevated SM levels are found (Daughaday et al. 1959). However, experimental results have been reported in which normal or high growth hormone concentrations and low somatomedin concentrations were measured.

In streptozotocin diabetic rats serum somatomedin decreased within 48 h to hypopituitary levels, and could not be restored by the administration of high doses of bovine growth hormone. Insulin injections to the animals either 24 or 48 h after streptozotocin prevented the decrease or normalized the low SM concentration (Philips & Young 1976; Philips & Orawski 1977). In dogs NSILA-s, another growth factor of the somatomedin family, decreased within 2 days of pancreatectomy and remained low for another 2 weeks despite serum growth hormone concentrations ten-times higher than normal. Initiation of insulin therapy in the hyperglycaemic dogs elevated NSILA-s within 8 days to pre-operative concentrations (Eigenmann et al. 1977). The results seem to indicate that insulin may be one regulator of the serum somatomedin concentration.

In the present experiments one normal and 2 hypophysectomized groups of rats were studied. Removal of the pituitary gland and subsequent solvent injections decreased the serum SM remarkably, confirming previous reports (Kaufmann et al. 1978; Takano et al. 1978), whereas treatment of the hypophysectomized animals with the sulphonylurea drug glibenclamide returned the serum somatomedin concentration to the normal range.

For the rat the minimal effective iv dose of glibenclamide was 0.1 mg/kg body weight (Schmidt et al. 1969). In the present study 10 times that dose was injected ip into the hypophysectomized rats. In rabbits the serum half-life of the drug was 7.4 h (Christ et al. 1969) and in dogs the blood glucose lowering activity of 0.2 mg/kg body weight glibenclamide continued for more than 24 h (Loubatières et al. 1969). These documented long lasting effects of glibenclamide in other species may explain the observation in the present study that serum insulin was significantly augmented 24 h after injection of the drug in hypophysectomized compared to normal rats.

Glibenclamide stimulates the secretion of insulin from the B-cells and insulin may have increased the generation of SM. However, the fasting insulin concentrations were extremely low in the hypophysectomized rats irrespective of whether the animals were treated with solvent or glibenclamide.

These results are in general agreement with previous reports in which the basal insulin concentrations in hypophysectomized rats amounted 15 ± 2 and 8 ± 1 μU/ml. Furthermore, the first phase of insulin release during an oral glucose tolerance test and both phases of the hormonal response during an iv glucose or iv glibenclamide test were greatly diminished (Penhos et al. 1971; Heinze et al. 1981).

Despite the low basal serum insulin concentrations even in the glibenclamide treated rats the results suggest that the experimental design of the present study favoured an augmented biological effect of the hormone, expressed as a normal serum SM concentration. This assumption is supported by a previous report which showed that iv injection of glibenclamide decreased the blood glucose to 29 mg/100 ml in conscious hypophysectomized rats compared to 99 mg/100 ml in control animals. During the test insulin release in the hypophysectomized animals was greatly diminished (Heinze et al. 1981). Both growth hormone deficiency (Soman et al. 1978) and glibenclamide treatment (Beck-Nielsen et al. 1979) enhance the binding of insulin to its target cells, thereby increasing the biological effect of insulin.

At present, it is unknown if glibenclamide itself without the action of insulin can increase the generation of SM.

The morphological changes observed in the 3 groups of rats deserve further comment. In the hypophysectomized animals glibenclamide, when compared with solvent treated animals, significantly stimulated skeletal growth as judged by the tibia test and by the X-ray study. But the effect was rather small compared to the controls, despite a normal serum somatomedin concentration. Thus the lack of growth factors others than the somatomedins may have contributed to the stunted growth.
Thyroxine, which was very low in the hypophysectomized animals, clearly stimulates postnatal skeletal growth in the rat. In high doses of 40 ng/g/day, T₄ completely normalized the growth rate of hypophysectomized rats (Glasscock & Nicoll 1981).

Low concentrations of insulin may also have prevented appropriate bone growth in the glibenclamide treated hypophysectomized rats. This assumption could explain why Salter & Best (1953) obtained an excellent growth promoting effect, when the hypophysectomized rats were injected with a long acting insulin preparation. Presumably, in their experiments both insulin and somatomedin were normal whereas in the present study glibenclamide treatment elevated the serum somatomedin concentration while insulin remained lower than normal.

Preliminary results seem to support this conclusion. The experiments of Salter & Best (1953) were repeated under identical conditions. Insulin treatment resulted in an augmented skeletal growth. This was measured with the tibia test and the incorporation of radioactive sulphate into rib cartilage of hypophysectomized rats. The increase in skeletal growth was comparable to the stimulating effect of 100 μg human growth hormone. The serum insulin concentrations 20 h after the last insulin injection amounted to 88 ± 18 μU/ml in the hypophysectomized compared to 52 ± 4 μU/ml in the sham operated control rats (Heinze et al. 1981).

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References


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