Growth hormone restores normal growth in selenium-treated rats without increase in circulating somatmedin C

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Abstract. Selenium intake (5.0 ppm) induces growth retardation, accumulation of selenium in somatotrophs, lack of growth hormone response to GHRH and an 80 per cent reduction in serum somatomedin C in infant rats. In addition, it induces a slight reduction in serum albumin and occasionally slight central liver necrosis. In order to determine the role of insufficient growth hormone production, the influence of exogenous growth hormone was studied during selenium intake in groups of rats during 25 to 46 days of age (post-weaning). A dosage of human growth hormone (100 μg twice daily) was chosen, this being sufficient to restore normal growth rate and normal serum somatomedin C in hypophysectomized rats of similar age. The weight gain in selenium-treated (3.3 ppm) rats was 46.0 ± 11.7 g (SD)/21 days, whereas in selenium rats given growth hormone it was 66.6 ± 8.9 g (P = 0.01), which was similar to the gain in control rats 72.3 ± 6.9 g. The latter two were less than the weight gain in control rats given growth hormone: 91.5 ± 11.5 g (P = 0.01 and P < 0.01). Serum somatomedin C in untreated rats was 151 ± 66 (SD) μg/l (25 days) increasing to 532 ± 91 μg/l (34 days) and 482 ± 64 μg/l (46 days). It did not increase above these levels in control rats given growth hormone. In selenium-treated rats, no increase occurred during growth hormone administration 138 ± 148 μg/l (34 days) and 185 ± 84 μg/l (46 days) (P < 0.001 vs untreated controls). Furthermore, there was no reduction in serum protein or albumin and no liver necrosis in these selenium-treated rats. As exogenous administration of growth hormone normalizes weight gain, the results indicate that growth retardation is at least partly due to insufficient growth hormone production. The increased growth rate in selenium-treated and control rats given growth hormone appears to be independent or circulating somatomedin C. This may be a direct effect of growth hormone or, more likely, may be caused by GH stimulation of peripheral (paracrine) formation of growth factors, possibly somatomedin C.

Although selenium is an essential trace element (Schwarz & Foltz 1957; Rotruck et al. 1973), it is also a most toxic agent when recommended doses are exceeded and these are not firmly established in animals and man. Selenium has acute toxic effects characterized by impaired CNS function, heart failure, and liver necrosis, but may also induce two forms of more chronic toxicity, depending on exposure (Shamberger 1983a). ‘Alkali disease’ is seen in animals exposed to 5–40 ppm of selenium in food or drinking water (Franke & Potter 1935; Moxon 1937). In young rats, a striking phenomenon is growth retardation, but anaemia and liver necrosis may also be observed (Franke & Potter 1935; Moxon 1937; Halverson et al. 1966; Palmer & Olson 1974). The other chronic toxicity, ‘blind staggers disease’, is rare and seen in livestock after consumption of selenium-containing plants, resulting in very high exposure (40–10 000 ppm) (Shamberger 1983a).

We demonstrated previously that selenium accumulates in the secretory granules of the somatomedins in vivo rat liver and mammary gland (Thorlacius-Ussing et al. 1981). Somatomedins are insulin-like growth factors that stimulate the growth of liver, bone, and muscle. Selenium is an essential trace element for these cells. However, selenium is also toxic at high doses, inducing liver necrosis and other toxic effects. These effects are mediated by the somatomedins, which are induced by growth hormone and other hormones. Selenium may interact with these hormones to cause growth retardation, as shown in this study.
troph (Thorlacius-Ussing & Danscher 1985). Also, very recently, we have shown that GH after stimulation with growth hormone releasing hormone (GRF40) and plasma somatomedin C (SMC) were both drastically reduced in animals treated with 15 ppm sodium selenite (5.0 ppm selenium) in the drinking water (Thorlacius-Ussing et al. 1987).

These results clearly indicated that the retardation in growth was due to accumulation of selenium in the pituitary, leading primarily to insufficient GH secretion and secondarily to insufficient somatomedin C formation. We suggested that reduced GH formation and release were due to attempted incorporation of selenium into its sulphur containing amino acids.

Such an explanation, however, was considered rather too simple. First, slight central necrosis was found in parts of the livers of some selenium-treated animals. Second, plasma proteins, in particular albumin, were also slightly but significantly reduced. Both factors suggested that other mechanisms may participate in the growth retardation. We decided to see, therefore, if GH administration could prevent growth reduction in selenium-treated rats. Human growth hormone was given in daily doses capable of restoring normal plasma SMC and normal growth rate in hypophysectomized rats.

Materials and Methods

In the first part of the study, 24 female Wistar rats were used. They were born at the institute and were the offspring from six pregnant rats (Møllegaards Avslab, Eiby, Denmark). Twenty-five days old female pups were randomized into four groups, each group of six being housed in a separate cage in a room with a 12:12 h artificial light cycle (06.00–18.00 h), temperature 21 ± 2°C, and humidity 55 ± 2%. All rats had free access to Altromin 1324® food (Altromin Specialfutterwerke, Lange, FRG) and water ad libitum.

Groups 1 and 2 were exposed to drinking water containing 10 mg of sodium selenite (Na2SeO3·5H2O) per litre (3.3 ppm selenium), whereas groups 3 and 4 had tap water. Rats in groups 2 and 4 were injected sc twice daily (07.00 and 16.00 h) with 100 µg of biosynthetic hGH (Nordisk Gentofte A/S, Denmark), the controls in groups 1 and 3 being injected with an equal volume of saline. Body weights were recorded prior to treatment and thereafter every second day. It was recently confirmed that weight gain in growing rats is a more precise indicator of growth than the tibial test (Jørgensen 1987). Weights were identical at onset of the experiment.

Blood was sampled in all rats when 34 and 46 days old. The first sample (0.7 ml) was collected from the retrobulbar venous plexus through a heparinized capillary tube. At end of the study, the animals were anaesthetized with sodium barbital (50 mg/kg) ip and 4–5 ml of blood collected by cardiac puncture before decapitation. Blood was sampled between 14.00 and 16.00 h, centrifuged and serum frozen for subsequent RIA and electrophoresis.

Hypophysectomized rats

In order to elucidate the dose response relation between hGH and effect on growth and somatomedin C levels, hypophysectomized rats were given various doses of hGH. Female Wistar/Mol rats, 28 days old, were hypophysectomized by a transauricular method (Illhardt 1971). Body weight was noted just prior to operation and again 14 days later. Rats whose weight either increased more than 10 g or decreased more than 4 g were excluded. Fifty-four rats were randomly divided into nine groups of six and injected twice daily (07.00, 16.00 h) with saline or hGH in 24-h dosages from 3.25 to 64 µg. After 10 days' hGH treatment, blood was collected for SMC assay. Growth rate was followed during GH administration.

Somatomedin C radioimmunoassay

The assay was performed using unextracted serum in dilutions 1:80 and 1:160 (1:20 and 1:40 for hypophysectomized rats). Identical values, but larger scatter, were obtained using acetic acid/methanol extracts. Somatomedin-binding serum protein seemed not to interfere at these dilutions as they gave identical estimations and as hypophysectomized rats had very low levels. Further, dilution experiments give expected results (Thorlacius-Ussing et al. 1987). Standards (0.5–10 µg/l) and 125I-somatomedin C were made up using a full sequence biosynthetic peptide (Amgen Biologicals, CA). Somatomedin C antibody C549/805C (raised by L. E. Underwood and J. J. van Wyk, University of North Carolina) was donated by the National Hormone and Pituitary Program. Standard or diluted serum, 100 µl, 125I-somatomedin C, 100 µl, and antisomatotropin C 1:40 000, 100 µl, were incubated for 48 h at 4°C, after which was added 100 µl of newborn calf serum and immediately thereafter 1700 µl PEG 6000 with 0.5% Tween 20 (Merck, Darmstadt, FRG). Following centrifugation (3000 rpm for 40 min at 4°C), supernatants were decanted and free and bound labels counted for calculation of binding percentage, a blank obtained by omission of antisomatotropin C being used for standardization. All sera were checked for antibody formation against hGH by addition of 125I-hGH, followed by incubation and paper chromatography. No detectable levels were found.

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Statistics
Means were compared by two factor analysis of variance using the Bonferroni test.

Liver histology
Sections were selected from each animal and stained with eosin/hematoxylin before light microscopy.

Results

Growth rate in selenium-treated rats
GH restores normal growth in selenium-treated rats. Whereas the selenium-treated rats had a weight gain of 46.0 ± 11.7 (SD) g during the three weeks' exposure, those rats also treated with hGH gained 66.6 ± 8.9 g (P = 0.007). No significant difference was observed between this latter group and the controls injected with saline, whose weight gain was 72.3 ± 6.9 g (P = 0.24). Those controls injected with the same dose of hGH had, however, a significantly higher weight gain (91.3 ± 11.5 g) than the control group given saline alone and the group treated with both selenium and hGH (P = 0.002 and P = 0.008) (Fig. 1). The initial weights (day 24) of the four groups were: selenium rats 44.2 ± 6.3 g (SD); selenium + GH rats 41.0 ± 11.0 g; control rats 48.7 ± 6.5; control + GH rats 42.3 ± 6.4 g.

Growth rate in hypophysectomized rats
Fig. 2 demonstrates that a 24-h dosage of 32 µg of hGH restores the weight gain rate of hypophysectomized rats to the rate of normal rats of the same age but at a lower level of weight. The fact that the rats did not exhibit a catch-up spurt of growth may be due to lack of especially thyroid hormones. Serum T3 was measured (data not shown) and found to be a fifth of normal in all hypophysectomized rats at the end of GH administration.

The dose-response curve is shown in Fig. 3. The curve is steep at low doses and then levels off with increased doses. The saline-treated rats gained 2.2 ± 2.0 g (SD) during the treatment period. The log dose response line is also depicted in Fig. 3. The data are fitted to a straight line by linear regression (applying the least square method), the correlation coefficient being r = 0.995.
**Somatomedin results**

In Fig. 4, serum SMC in hypophysectomized rats is shown after exposure to different doses of hGH. A clear-cut dose-response curve is observed. Untreated hypophysectomized rats had a plasma SMC level of 25 µg/l, whereas after exposure to 32 µg of hGH, plasma SMC levels increased to 400 µg/l. Doubling the dose to 64 µg resulted in serum SMC of 480 µg/l only, indicating that further increase would require huge GH administration. In fact, the dose-response curve is rectilinear in a semi-log scale. The ‘maximum’ value of approximately 500 µg/l is identical to the average SMC value in untreated controls, Fig. 5 (group 3).

While hGH could thus normalize the SMC production in hypophysectomized rats, it appears unable to induce any increase in SMC in selenium-treated animals (Fig. 5). Although selenium rats treated with hGH have significantly higher and normal growth rates, no difference was found in the plasma SMC level between GH and salinetreated selenium rats either at day 34 or at day 46 ($P = 0.59$ and $P = 0.75$).

In order to determine any age-dependent change in SMC production during the period relevant in our study (25–46 days), plasma SMC was estimated in 15 female Wistar rats at the age of 25 days. This was found to be 151 ± 66 µg/l, and so SMC production appeared to rise during the interval between 25 and 34 days to a level of about 500 µg/l (Fig. 5). Thus, plasma SMC was maintained at the initial low 25 day level during selenium administration.

Serum protein at age 46 days in the four groups was as follows: selenium rats 49 g/l ± 5.3 (SD); selenium + GH rats 44 g/l ± 2.7 ($P < 0.05$); control rats 51 g/l ± 4.3; and control rats + GH 44 g/l ± 3.5 g/l ($P < 0.05$). The respective serum albumin for the four groups was: 29 ± 3.1; 26 ± 1.5; 31 ± 4.2; and 26 ± 3.7 g/l. It appears that selenium had not altered the serum levels in the present study, whereas GH treatment apparently induced a slight reduction in serum protein in both selenium and control rats. The serum electrophoresis showed that this result was due principally to reduction in serum albumin.

**Histology**

There were no light microscopically visible lesions, and, moreover no signs of necrosis in any of the liver sections.
HUMAN GROWTH HORMONE, DAILY DOSE μg

*Fig. 4.*

Left part: serum somatomedin C immunoreactivity ± SD in hypophysectomized rats (N = 6) after 10 days' treatment with either saline or hGH in total daily doses from 3.25 to 64 μg. Right part: as previously but in log scale.

Serum somatomedin C immunoreactivity in untreated rats at start of experiment (day 25, N = 15) and at day 34 and 46 after selenium and saline treatment (group 1, N = 6) selenium and hGH treatment (group 2, N = 6) saline treatment (group 3, N = 6), and hGH treatment (100 μg twice daily, group 4, N = 6).

*Fig. 5.*
Discussion

This study shows that growth hormone administration restores growth in post-weaning selenium-treated rats, which points to selenium inhibition at the pituitary level, resulting in growth hormone deficiency. It is, however, interesting that selenium apparently also induces an independent inhibition of the SMC production, as the animals in the two selenium-treated groups had very low plasma levels irrespective of hGH treatment. A possible explanation is an attempted incorporation of selenium instead of sulphur in either SMC or the enzymes responsible for its production, as the metabolisms of selenium and sulphur have much in common (Shamberger 1983b). Another possibility is an oxidation of sulphur groups in these proteins, as selenium is known to exert such a process. Indeed, this has been suggested to be the major toxic effect of selenium (Shamberger 1983a).

If one of these suggestions were correct, it might imply a reduction in formation of several sulphur-containing peptides and proteins generated in those tissues especially accumulating selenium (e.g. liver). This would be in accordance with our previous observation (Thorlacius-Ussing et al. 1987) of reduced serum protein and albumin in selenium-treated rats. In the present study no such reduction was seen, probably because a slightly lower selenium dose was given (3.3 vs 5.0 ppm) and for a shorter period (post weaning only vs from birth). Furthermore, no histological evidence of liver necrosis was observed in this study.

The ability of hGH to restore growth in selenium-treated post-weaning rats without increase in plasma SMC is, at first sight, not in accordance with the somatomedin hypothesis proposed by Salmon & Daughaday (1957) and Daughaday et al. (1972). It concurs, however, with reports that local administration of hGH and rGH into the cartilage growth plate of the proximal tibia accelerates longitudinal bone growth (Isaksson et al. 1982; Russel & Spencer 1985) and that hGH stimulates proliferation of cultured chondrocytes (Madsen et al. 1983). Further, chondrocytes have specific binding sites for GH (Eden et al. 1983). In the experiments mentioned, the possibility of local formation of SMC cannot be excluded. Evidence that growth hormone induces local synthesis in the kidney, lung, heart and testes was supplied recently by a study of D’Ercole et al. (1984) who found increases in tissue extractable somatomedin C in GH-treated hypophysectomized rats. If local SMC generation in response to the hGH treatment is responsible for the restoration to normal growth in the present selenium rats, it appears to be insufficient to increase systemic SMC levels. Finally, a possible explanation for the reduced hepatic SMC in serum might be that the production of SMC-binding protein is inhibited, which would also reduce serum SMC levels and possibly its affinity for binding sites in tissues.

Recently, however, at the 1986 meeting of the American Endocrine Society, Schlechter et al. (1986) demonstrated that rGH infused directly into the hepatic portal vein of rats was not more growth promoting than when infused into the jugular vein. Moreover, when rGH was infused intra-arterially into a hindlimb simultaneously with antiserum to somatomedin C, the growth effect on the tibial plate of GH infused unilaterally alone was totally abolished. The authors concluded that the growth promoting effect of GH on peripheral tissues is mediated primarily by locally produced somatomedin C and that the role of the liver is uncertain.

The present study demonstrates that in vivo body growth in rats at this stage of development also seems to be largely independent of blood-borne somatomedin C. The growth increase induced by hGH in selenium-treated as well as in control rats (approximately 20 g weight) occurred without discernable increase in plasma somatomedin C. The mechanism appears, therefore, to be due either to a direct effect of GH, or, considering the results of Schlechter et al. (1986), more probably to stimulation of local somatomedin C formation through a paracrine effect. A third possibility is that GH sensitizes immature cells to the action of somatomedin C (circulating or locally synthesized) as it has recently been demonstrated by Zezulak & Green (1986) in vitro in pre-adipocyte cultures. The GH-treated selenium rats did not reach the weight gain of GH-treated controls, and so some inhibition of peripheral synthesis of somatomedin may occur in selenium-treated rats.

In the hypophysectomized rats, hGH increased plasma SMC in a dose-dependent manner until further increase became very small at normal levels around 500 µg/l after injection of 32–64 µg of hGH twice daily. In the selenium study, we more than tripled the dose, indicating that lack of
SMC production is not due to insufficient stimulation. This study supports the notion that SMC production is age-dependent, as an impressive increase in SMC from about 150 µg/l post weaning to about 500 µg/l between day 25 and 34 was observed, whereas no further increase was obtained during the remaining experimental period which lasted until day 46. This level is close to that observed in the hypophysectomized rats exposed to 62 µg of hGHI, the particular dosage that restored weight gain to normal. The control rats at this age have probably reached a SMC production which requires very large growth hormone doses to increase significantly (Fig. 4).

In conclusion, these results indicate that growth retardation in selenium-treated rats is due primarily to insufficient growth hormone production, as this retardation is overcome by administration of exogenous GH. Incidentally, we found indications that hepatic somatomedin C production is also directly inhibited during selenium intake, and also that normal growth rate may be restored without increase in serum SMC immunoreactivity, but through other effects of GH, probably through stimulation of somatomedin formation in the periphery. The results thus also suggest that the growth retardation observed in rats exposed to moderate selenium intake is not due to anorexia or gross liver damage. The inhibited production of sulphur-containing peptides such as hepatic somatomedin C and GH at a vulnerable period of life is in accordance with the finding of particularly great accumulations of selenium in liver and somatotrophs (Shamberger 1983a).

Selenium is becoming increasingly popular as an ingredient in vitamin/mineral tablets and in alternative medicine recipes, because of a cancer prophylactic effect which is undocumented in man. The growth retarding effect of selenium and the observation of severely reduced GH and hepatic SMC production in infant rats highlight the need for human studies.

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