ACTIONS OF GROWTH HORMONE, PROLACTIN AND THYROID HORMONE ON SERUM SOMATOMEDIN-LIKE ACTIVITY AND GROWTH IN HYPOPITUITARY DWARF MICE

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(Received 19 October 1976)

SUMMARY

Hypopituitary dwarf mice (Snell's strain) were found to have much reduced levels of serum somatomedin when compared with normal mice (apparently normal members of the Snell strain). Treatment with bovine growth hormone, prolactin or thyroxine induced growth in these animals; this was accompanied in each case by increased levels of serum somatomedin (primarily somatomedin C). Growth hormone had a dose-dependent growth-promoting effect, but this was not reflected in dose-dependent increases in serum somatomedin levels. These results are in accordance with the concept that somatomedin is involved in the regulation of overall somatic growth, but it seems likely that other factors are also involved.

INTRODUCTION

Many of the actions of pituitary growth hormone on the metabolism of cartilage and other tissues are mediated by somatomedins (reviewed by Hall & Van Wyk, 1974), but it has not yet been established whether these factors play a role in all the physiological actions of growth hormone. In particular, the role of somatomedins in regulating general somatic growth has not been elucidated.

The hereditary dwarf mouse (Snell's strain; Snell, 1929), which has a defective pituitary gland secreting only small quantities of several pituitary hormones, provides a useful model for studies on the action of growth-promoting hormones (Fønss-Bech, 1947; Bartke, 1965; Wallis & Dew, 1973) and can also be used for bioassay of growth hormone. Here we have used the Snell strain to investigate the role of somatomedins in somatic growth. The effects of growth hormone, prolactin and thyroxine on body weight and somatomedin levels were investigated in an attempt to discover to what extent growth and somatomedin are correlated. The assay system used to detect somatomedin-like activity is thought to measure primarily somatomedin C, but since it is possible that more than one somatomedin is active in the assay and in general somatic growth, we have retained the more general term somatomedin. A brief account of some of this work has been presented elsewhere (Holder & Wallis, 1976).

MATERIALS AND METHODS

Dwarf mice

Animals were reared as described by Wallis & Dew (1973). The dwarf mice are infertile double recessives (dw/dw), bred from normal mice heterozygous for the dw gene. For the

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experiments described here the dwarf mice were aged 8–12 weeks and weighed 6–10 g (at the beginning of the experiment). No differences have been detected in the responses of male and female dwarf mice and these were therefore pooled and used at random. Since the starting weights of the mice were restricted, analysis of covariance to correct for large differences in body weight was unnecessary.

**Hormones**

Bovine growth hormone (NIH-GH-B17; 0.92 i.u./mg) and bovine prolactin (NIH-P-B3; 24·1 i.u./mg) were a gift from the Endocrinology Study Section of the National Institutes of Health, Bethesda, Maryland, U.S.A. Sodium-1-thyroxine was obtained from Glaxo Laboratories.

**Preparation and injection of solutions**

Solutions of hormones for injection were prepared in 0·9 % NaCl as described by Wallis & Dew (1973). Fresh solutions were prepared every 4–6 days. Animals were weighed and injected daily (0·1 ml; subcutaneously in the back). Treatment was continued for 25 days and any increase in body weight during this period was taken as the growth response (for each animal, the difference between the mean weight for 3 days before, and including, the day of the first injection and the final weight, on day 26). Control animals were injected with saline.

The animals were allocated to treatment groups at random. Animals were distributed among several small cages, each cage containing a representative of each treatment.

**Serum samples**

After injection, blood samples were obtained from the mice by cardiac puncture under ether anaesthesia. Sufficient blood (0·3–0·4 ml) was obtained from each animal to yield 150–200 µl serum. The blood was allowed to clot at room temperature, centrifuged on a bench centrifuge for 20 min, and the serum was decanted.

**Somatomedin-like activity**

This was detected by the stimulation of uptake of radioactive sulphate by normal rat costal cartilage. No attempt has been made to equate levels of serum sulphating activity with absolute somatomedin levels because the assay may be detecting more than one somatomedin (although somatomedin C is probably the main form detected).

Normal male Sprague-Dawley rats, 22–24 days old, were starved for 48 h before death. Costal cartilages from ribs 5–7 were removed (discarding the distal 5 mm of each segment; Yde, 1968) and cut into 2 mm sections. These cartilage sections (about 16/rat) were distributed among sterile tubes containing ice-cooled, sterile 0·9 % NaCl until dissection of all the animals was complete.

Each pool of cartilage sections was then transferred to a sterile 10 ml conical flask containing 5 ml sterile Krebs-phosphosaline buffer with 2 mg glucose/ml, supplementary amino acids, 50 units penicillin and 50 µg streptomycin/ml (Daughaday & Reeder, 1966). The cartilage sections were pre-incubated in this medium for 24 h at 37 °C. After pre-incubation, four cartilage sections were incubated with each of the dwarf mouse serum samples for 48 h, the last 24 h in the presence of 2 µCi 35SO4/ml (obtained as sulphate in aqueous carrier-free solution, pH 6–8, from The Radiochemical Centre, Amersham, Bucks). The incubation volume was 2 ml, and 5 % serum samples were used throughout.

After incubation the cartilage segments were immersed in boiling water for 10 min, soaked overnight in saturated sodium sulphate, and washed in running tap water for 2 h and distilled water for 1 h. All adhering soft tissue was removed and, after drying for 1 h at
45 °C, the cartilage sections were weighed. The cartilage sections were then sealed in hydrolysis tubes with 200 μl 98% formic acid and heated at 110 °C for 1 h when solubilization was complete. The entire sample was added to 10 ml scintillation fluid (0.5% 2,5-diphenyloxazole, 30% Triton X-100, 70% toluene; w/v/v) and the radioactivity was measured in a Beckman LS 233 scintillation counter. Incorporation of radioactive sulphate was expressed as d.p.m./mg cartilage.

No attempt was made to correct for the sulphate content of the serum samples added to the medium. This was considered unnecessary because the concentration of serum used throughout was 5%, so that the dilution of sulphate concentration in the medium was constant (and small). The concentration of sulphate in the medium was much greater than that in 5% serum so that fluctuations in serum sulphate concentration should have been effectively swamped (Audhya & Gibson, 1974; Van den Brande & Du Caju, 1974).

RESULTS

Somatomedin-like activity in the serum of normal mice and rats, and dwarf mice

The small size of dwarf mice is attributable to lack of growth hormone and possibly other anabolic hormones. It seemed likely that this would be reflected by low levels of serum somatomedin. Five per cent serum samples (a submaximal stimulating concentration) from dwarf mice, normal mice (apparently normal members of the Snell strain) and rats (Sprague–Dawley strain) were therefore assayed for serum somatomedin-like activity. Incorporation of radioactive sulphate into cartilage in the presence of 5% dwarf mouse serum (15170 ± 769 (S.E.M.) d.p.m./mg cartilage; six observations) was not significantly greater than incorporation in medium alone (13000 ± 991 d.p.m./mg cartilage; six observations). Incorporation in the presence of 5% normal mouse serum (29979 ± 1014 d.p.m./mg cartilage; ten observations) and rat serum (32061 ± 1486; seven observations) was significantly greater than that in medium alone (P < 0.001 in each case). The reduced growth and lowered hormonal levels of dwarf mice are thus accompanied by much reduced levels of serum somatomedin.

Effect of bovine growth hormone on body weight and serum somatomedin-like activity in dwarf mice

Figure 1 illustrates the effects of three doses of bovine growth hormone (5, 20 and 80 μg/day), injected for 25 days, on body weight and serum somatomedin-like activity in dwarf mice. All doses of growth hormone promoted a significant growth response. A linear relationship was found between the increase in body weight and the log dose of hormone administered, in agreement with earlier findings (Fønss-Bech, 1947; Wallis & Dew, 1973).

Serum somatomedin-like activity in dwarf mice injected with bovine growth hormone was significantly higher than that in animals injected with 0.9% NaCl, although not as high as that in normal mice (Fig. 1). The dose dependency of the growth response was not reflected in the levels of serum somatomedin-like activity. Thus, the growth-promoting effect of bovine growth hormone on dwarf mice was accompanied by increased serum somatomedin-like activity.

Effect of bovine prolactin on body weight and serum somatomedin-like activity of dwarf mice

Also shown in Fig. 1 are the effects of three doses of bovine prolactin (5, 20 and 80 μg/day), injected for a period of 25 days, on body weight and serum somatomedin levels of dwarf mice. Both 20 and 80 μg prolactin/day promoted a significant growth response. The response for prolactin was less than that for growth hormone, and a clear dose–response relationship was not established; the results agree well with those of Wallis & Dew (1973).
Fig. 1. Growth response (a) and serum somatomedin-like activity (b) in dwarf mice treated with growth hormone or prolactin. Mice were injected with growth hormone, prolactin or saline daily, for 25 days, and growth response and serum somatomedin-like activity (5% serum) were determined. Values are means ± S.E.M. and the number of animals in each treatment group is given in parentheses. Serum somatomedin-like activity of untreated normal mice (NM) and the sulphate incorporation occurring in medium alone (MED) are also shown. *P < 0.05; **P < 0.01; ***P < 0.001: compared with saline-injected control mice.

Serum somatomedin-like activity was significantly higher in all prolactin-treated animals than in animals treated with 0.9% NaCl. The levels of serum somatomedin-like activity resulting from prolactin injections were very similar to those obtained with the same doses of growth hormone, and the lower growth-promoting action of prolactin (compared with growth hormone) was thus not reflected by a significant reduction in the levels of serum somatomedin-like activity.

**Effect of thyroxine on body weight and serum somatomedin-like activity of dwarf mice**

The effect of three doses of thyroxine (2, 4 and 8 μg/day), injected for a period of 25 days, on body weight and serum somatomedin-like levels of dwarf mice is illustrated in Table 1. All doses promoted a significant growth response, which was not dose dependent. The weight increase was generally greater than that for growth hormone- and prolactin-treated animals. The results are in agreement with earlier studies (Wallis & Dew, 1973).

Somatomedin-like activity in serum of dwarf mice injected with thyroxine was significantly higher than in control animals although not as high as that in normal animals. The stimulation by serum from thyroxine-treated animals (30–40%) was rather lower than that caused by serum from growth hormone- or prolactin-treated animals (60–80%).
Somatomedin and growth in dwarf mice

Table 1. Growth and serum somatomedin-like activity (5 % serum) in dwarf mice treated with thyroxine

<table>
<thead>
<tr>
<th>Dose of thyroxine (µg/day for 25 days)</th>
<th>Increase in body weight (g)</th>
<th>35SO4 uptake (d.p.m. × 10^−3/mg cartilage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.26±0.12*** (9)</td>
<td>21.86±1.35** (9)</td>
</tr>
<tr>
<td>4</td>
<td>4.26±0.57** (9)</td>
<td>21.95±0.99** (8)</td>
</tr>
<tr>
<td>8</td>
<td>3.50±0.41* (8)</td>
<td>21.61±1.65* (7)</td>
</tr>
<tr>
<td>0 (saline controls)</td>
<td>1.95±0.40 (8)</td>
<td>16.08±1.27 (6)</td>
</tr>
</tbody>
</table>

Values are means ± s.e.m. and the number of animals in each treatment group is given in parentheses. The death of some animals during cardiac puncture accounts for the different number of animals in some groups.

* P < 0.05; ** P < 0.01; *** P < 0.001: compared with saline-injected control mice.

Direct in-vitro effect of growth hormone, prolactin and thyroxine on cartilage sulphation

The possibility that growth hormone, prolactin or thyroxine might be directly responsible for the sulphating activity of serum from treated dwarf mice was investigated. Three doses of each of these hormones were added directly to the assay medium and their direct effect on sulphation of cartilage was studied. In no case was there any significant stimulation of sulphation. The results for prolactin and growth hormone are in general agreement with those of previous workers using similar rat costal cartilage assays (Francis & Hill, 1975, using ovine prolactin; Salmon & Daughaday, 1957, using bovine growth hormone).

DISCUSSION

There is strong, though mainly circumstantial, evidence to support the view that the general growth-promoting actions of growth hormone are largely mediated by somatomedin. In particular it has been shown in several clinical studies that serum somatomedin levels correlate well with growth, and may, indeed, do so better than levels of growth hormone itself (reviewed by Hall & Van Wyk, 1974). On the other hand, attempts to induce somatic growth with somatomedin preparations have so far been unsuccessful (Uthne, 1975). Until a purified preparation of somatomedin has been shown to exhibit direct growth-promoting activity in vivo, its role in mediating the growth-promoting actions of growth hormone will remain unsubstantiated. The aim of the work described here was to assess to what extent induction of growth by various hormones in dwarf mice was associated with somatomedin-like activity.

Somatomedin-like activity in serum from untreated dwarf mice was very low. This is in agreement with the many observations of low levels in serum of hypophysectomized rats, and is presumably a consequence of the very low levels of several pituitary hormones, especially growth hormone, in the Snell dwarf mouse. Growth hormone induced growth in dwarf mice and also increased somatomedin-like activity; for the latter effect a dose dependency could not be demonstrated. The fact that growth hormone induces both growth and somatomedin-like activity agrees with a role for somatomedin in growth promotion, but the lack of dose dependency for the somatomedin response somewhat counteracts such a role. However, the lack of dose dependency may be a consequence of the experimental design used, since somatomedin-like activity was measured only at the end of a 25-day injection period. It may be that a dose dependency for somatomedin occurs at earlier stages of the injection schedule.

Prolactin also promoted growth of dwarf mice but was less effective than growth hormone; dose dependency was not established. Prolactin increased somatomedin-like activity in these animals, to an extent similar to that achieved with the same doses of growth hormone.
(despite the lower growth response). Association of increased somatomedin-like activity with increased growth agrees with the concept that the growth-promoting action of prolactin is mediated by somatomedin but, again, some details of the response raise doubts. We have proposed previously that prolactin and growth hormone share a common mechanism in promoting growth in dwarf mice (Wallis & Dew, 1973), and mediation of the effect by somatomedin could be such a mechanism. Our observations with prolactin on dwarf mice agree well with those of Francis & Hill (1975) who showed that perfusion of rat liver in vitro with medium containing ovine prolactin stimulated somatomedin production.

The growth-promoting effect of thyroxine in dwarf mice is well established and the results of the present study confirm those of previous workers. Overall, the response was greater than for growth hormone and prolactin. Somatomedin activity in serum of thyroxine-treated dwarf mice was significantly higher than that in serum of control animals, although the response appeared to be slightly smaller than with growth hormone or prolactin. In this case too, hormonally induced growth was associated with increased somatomedin-like activity, suggesting that somatomedin mediates the growth-promoting effects of thyroxine. However, the relatively low somatomedin response and relatively high growth response again throws some doubt on the simple concept that somatomedin alone is responsible for this growth. We have suggested previously (Wallis & Dew, 1973) that thyroxine and growth hormone operate through quite distinct sites in promoting growth in dwarf mice. The present results tend to contradict this idea, since a common mechanism (via somatomedin) may be involved. Gaspard, Wondergem & Klitgaard (1975) showed that thyroxine can promote body weight and somatomedin levels in hypophysectomized rats, and these results agree well with ours in dwarf mice. These authors suggested that thyroxine and growth hormone might act in part through different somatomedins and this might also explain some of the discrepancies in our results.

Our results agree with the concept that somatomedin is involved in regulation of overall somatic growth. The correlation between somatomedin and growth is not complete however. This may be partly a consequence of the experimental design, but it seems likely that it is also a consequence of the involvement of additional factors in growth promotion, or to the differential effects of several distinct somatomedins. The assay used in this work detects primarily somatomedin C, but it would be premature to conclude that this is the only somatomedin detected or the only one involved in general somatic growth.

We thank Mrs Molly Reed and Mr George Blank for expert management of the dwarf mouse colony. We are grateful to the Endocrinology Study Section of the National Institutes of Health for supplying some of the hormones used, and to the Medical Research Council for financial support.

REFERENCES


