EFFECT OF WITHDRAWAL OF GROWTH HORMONE ADMINISTRATION ON LONGITUDINAL BONE GROWTH IN THE HYPOPHYSECTOMIZED RAT

By
K.-G. Thorngren and L. I. Hansson

ABSTRACT

Bovine growth hormone was given daily for 10 days to female Sprague-Dawley rats hypophysectomized at the age of 60 days, beginning 15 days post-operatively. Longitudinal bone growth, studied in the proximal tibia, was reactivated and continued at an accelerating rate during the period of hormone administration and for a further 5 days after its withdrawal, but then ceased.

The effect of withdrawal of growth hormone on the width of the growth plate of proximal tibia, the size of its degenerative cells, and the weight of body and heart was also studied.

The cell production in the proximal growth plate of tibia was calculated. The changes in longitudinal bone growth were found to be due mainly to changes in cell production in the growth plate.

The growth in length gradually decreases after hypophysectomy (Simpson et al. 1950; Asling & Evans 1956; Baume et al. 1958; Yonaga 1969) and reaches a low basal growth rate dependent on the age at hypophysectomy (Thorngren et al. 1973a). Growth hormone is known to promote growth in length in the hypophysectomized rat (for review see Asling & Evans 1956; Asling et al.)

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1965; Evans et al. 1966; Urist 1972). This effect on the longitudinal bone growth is dependent on the dose and the length of the administration period (Thorngren et al. 1973b).

The present investigation was made to determine the effect of withdrawal of growth hormone administration on the longitudinal bone growth in the hypophysectomized rat. With tetracycline as an intravital marker of the longitudinal bone growth from the proximal growth plate of the tibia, this effect was determined during various periods after the cessation of growth hormone administration. The width of the growth plate of proximal tibia as well as the size of its degenerative cells, and the weight of body and heart were also studied to compare the changes in different growth parameters. The cell production in the proximal growth plate of the tibia was calculated to analyse whether the changes in longitudinal bone growth were due mainly to changes in the cell production or in the size of the degenerative cell in the growth plate.

**MATERIAL AND METHODS**

Female Sprague-Dawley rats with known date of birth (day of birth registered as day 1) were delivered from the breeding farm (Møllegaard, Denmark) one week before hypophysectomy. The animals were kept in clear plastic cages with a metal grill top in an air-conditioned room with daylight illumination. The temperature was 22–24°C and the relative humidity 40–50%. On arrival and during the experiments, the general condition of the animals was controlled and the weight was registered. All the animals were given pellets (Anticimex 213) and water *ad libitum*; 150 animals were hypophysectomized at 60 days of age; 70 survived the investigation period.

The rats were hypophysectomized by the parapharyngeal approach (Smith 1930; Thorngren et al. 1973a) and after a post-operative control period of 15 days, the administration of growth hormone was started. The animals were given one daily sc injection of 25 μg bovine growth hormone (NIH-GH-B15) obtained from National Institutes of Health, USA. This was dissolved in saline and a new solution prepared each day. Two groups of animals were given NIH-GH-B15 for 5 or 10 days and killed on the day after the last administration. Three other groups of animals were given growth hormone for 10 days and were thereafter kept for a further 5, 10 or 15 days before being killed. One group of control animals was killed 15 days post-operatively when the growth hormone administration for the other groups was started. The other control values were taken from Thorngren et al. (1973b).

To determine the accumulated growth in length, the experimental animals were given two consecutive doses (10 mg/kg) of oxytetracycline, OTC (Terramycin®), intraperitoneally, and were anaesthetized with ether to increase the accuracy of the injections (Hansson et al. 1972). The animals were given two injections of OTC, the first at hypophysectomy and the second 15 days post-operatively. After the administration of growth hormone and the observation period following withdrawal of the growth hormone administration, they were killed with ether. The accumulated growth in length was determined 0–15 days post-operatively and also during the whole period of growth hormone administration and the following withdrawal period. The group of animals killed 15 days post-operatively was only given one injection of OTC at
hypophysectomy to determine the accumulated growth in length 0–15 days post-operatively.

The proximal tibia of the right side was dissected out and undecalcified sections were made according to a previously described method (Hansson 1964, 1967) from the medial part of the proximal tibia (Hansson et al. 1972). The growth determination was made with a fluorescence microscope with an error of method calculated to about 5 µm (Hansson 1967; Hansson et al. 1972). The measurements were made between the two fluorescent bands corresponding to the injections of OTC, and between the second fluorescent band and the erosion line between metaphysis and growth plate (Thorngren et al. 1973b). The width of the second fluorescent band was also determined (Thorngren et al. 1973a). In the control animals killed 15 days post-operatively, the determination was made between the fluorescent band and the erosion line.

In some instances, fluorescent bands were missing at the microscopical determination, as shown in earlier investigations with the use of intraperitoneal injection of OTC (Hansson et al. 1972). In the present investigation, this was found in 7 animals, making it impossible to determine the post-operative accumulated growth in length during all the intended periods of observation. These animals were also excluded from the calculations of the other growth parameters.

Decalcified longitudinal and frontal sections of the left proximal tibia stained with haematoxylin and eosin were studied microscopically (Hansson 1967) with regard to the morphology of the growth plate. The growth plate was divided into different zones (Hansson 1967; Thorngren & Hansson, in press). The total width, the width of its undifferentiated zone and that of its columnar zone were determined as described by Hansson (1967). The size of the degenerative cell situated close to the metaphysis in the growth plate was determined (Thorngren & Hansson, in press). The accumulated cell production in the proximal growth plate of the tibia was calculated from the accumulated longitudinal bone growth divided by the size of the degenerative cell (Thorngren & Hansson, in press).

The completeness of the hypophysectomy was determined by microscopical examination of serial sections of the sella turcica (Jacobsohn 1966; Thorngren et al. 1973a) stained with azan. In addition, body weight and weight of heart ventricles (in the following called heart weight) were registered. Of the operated animals, 29 % (44/150) survived with complete hypophysectomy. The total of operated animals minus those surviving with incomplete hypophysectomy shows the survival to be 36 % (44/124).

RESULTS

Growth in length 0–15 days post-operatively

The determination of accumulated growth in length of proximal tibia and width of the second fluorescent band after the post-operative control period of 15 days allowed a separation of animals with complete and incomplete hypophysectomy, as shown in earlier investigations (Thorngren et al. 1973a,b; Thorngren & Hansson 1973a). The animals with incomplete hypophysectomy had higher values both for longitudinal bone growth and width of the fluorescent band. The results from the animals with complete hypophysectomy are presented in the following.
Effect of administration and withdrawal of growth hormone

Growth in length. – After the administration of 25 \( \mu \text{g} \) NIH-GH-B15 daily for 5 or 10 days the accumulated growth in length of the proximal tibia increased with the period of administration. Animals given growth hormone had higher longitudinal bone growth than the controls. In the animals kept for various periods after the 10-day administration of growth hormone, the accumulated growth in length was significantly higher than in animals killed on the day after the last injection of growth hormone (Fig. 1, Table 1). The accumulated growth in length reached a maximum 5 days after cessation of the growth hormone administration and then remained constant. Further increase in the period to 10 and 15 days did not result in any increase in the growth in length (Fig. 1, Table 1).

The mean daily growth rate for the post-operative periods can be calculated from the values of accumulated growth in length for the various groups in Table 1. During the periods of hormone administration the growth rate continued to accelerate (8 and 14 \( \mu \text{m/day} \) during 15–20 and 20–25 days post-operatively, respectively). Then the growth rate increased further and the maximum growth rate (38 \( \mu \text{m/day} \)) was found during the 25–30 days post-operative period, i.e. during the 5 days after stopping growth hormone administration. During the following post-operative periods after stopping growth hormone administration, the growth in length had ceased.

Size of degenerative cell. – After the administration of growth hormone daily for 5 or 10 days, the size of the degenerative cell increased during 5 days administration and then remained constant after 10 days administration of growth hormone (Fig. 1, Table 1). Animals given growth hormone had bigger cells than the controls. During the withdrawal period after growth hormone administration, the cell size decreased somewhat (Fig. 1, Table 1).

Cell production. – The accumulated cell production calculated from the accumulated longitudinal bone growth and the size of the degenerative cell showed changes similar to those described for the growth in length (Fig. 1, Table 1).

Cartilage width. – The total width of the growth plate of proximal tibia increased after 5 days administration of growth hormone. After additional 5 days administration the width decreased somewhat (Fig. 2, Table 1). The controls had an almost constant width of the growth plate. During the 5-day withdrawal period after the growth hormone administration, the width remained unchanged as compared with the period of administration. Thereafter, it decreased somewhat when the withdrawal period was increased to 10 and 15 days (Fig. 2, Table 1). The undifferentiated zone was almost constant in
Fig. 1.

Accumulated longitudinal bone growth (top), size of degenerative cell (middle), and calculated accumulated cell production (below). Proximal tibia in female rats hypophysectomized at age 60 days, complete hypophysectomy. Daily administration of 25 µg growth hormone beginning 15 days post-operatively (——), withdrawal period (-----), controls (------). Mean value and standard deviation.

all groups. The width of the columnar zone changed in parallel with the total cartilage width (Table 1).

Body weight. – After the administration of growth hormone for 5 days, the body weight was unchanged as compared with the body weight at the start of
Table 1.
Effect of growth hormone (NIH-GH-B15) given daily to female rats hypophysectomized at 60 days of age, beginning 15 days postoperatively. Administration for 5 or 10 days (25 µg x 5 or 25 µg x 10 in column Dose x time). Administration of growth hormone for 10 days was followed by a withdrawal period of 5, 10 or 15 days (25 µg x 10 + 5,

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose x time (µg x days + days)</th>
<th>Number of animals</th>
<th>Accumulated growth in length (µm)</th>
<th>Size of degenerative cell (µm)</th>
<th>Accumulated cell production (cells)</th>
</tr>
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<tbody>
<tr>
<td>I</td>
<td>0 x 0</td>
<td>14</td>
<td>0</td>
<td>18 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>II</td>
<td>0 x 10¹</td>
<td>8</td>
<td>14 ± 19</td>
<td>17 ± 2</td>
<td>0.8 ± 1.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>I⁺</td>
<td>I⁺</td>
</tr>
<tr>
<td>III</td>
<td>0 x 20¹</td>
<td>14</td>
<td>43 ± 33</td>
<td>16 ± 1</td>
<td>2.7 ± 2.1</td>
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<td></td>
<td>II⁺</td>
<td>II⁺</td>
</tr>
<tr>
<td>IV</td>
<td>0 x 30¹</td>
<td>7</td>
<td>22 ± 23</td>
<td>14 ± 1</td>
<td>1.6 ± 1.6</td>
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<td></td>
<td></td>
<td></td>
<td>III⁻</td>
<td>III⁻</td>
</tr>
<tr>
<td>V</td>
<td>25 x 5</td>
<td>6</td>
<td>39 ± 26</td>
<td>22 ± 3</td>
<td>1.8 ± 1.2</td>
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<td></td>
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<td>I⁺⁺⁺⁺, II⁻</td>
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<td>VI</td>
<td>25 x 10</td>
<td>5</td>
<td>107 ± 25</td>
<td>22 ± 3</td>
<td>4.9 ± 1.7</td>
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<td>V⁺⁺⁺⁺, II⁺⁺⁺⁺</td>
</tr>
<tr>
<td>VII</td>
<td>25 x 10 + 5</td>
<td>5</td>
<td>298 ± 87</td>
<td>21 ± 1</td>
<td>14.2 ± 4.7</td>
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<td>6</td>
<td>273 ± 52</td>
<td>20 ± 1</td>
<td>13.4 ± 2.5</td>
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<td>VII⁻, III⁺⁺⁺⁺</td>
</tr>
<tr>
<td>IX</td>
<td>25 x 10 + 15</td>
<td>6</td>
<td>264 ± 66</td>
<td>20 ± 1</td>
<td>13.4 ± 3.2</td>
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<td>VIII⁻, IV⁺⁺⁺⁺</td>
<td>VIII⁻, IV⁺⁺⁺⁺</td>
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</table>

Values = means ± standard deviations.
1) Values from Thorngren et al. (1973b).

the administration. After 10 days hormone administration, the body weight had increased and was higher than in the controls (Fig. 2, Table 1). During the 5-day withdrawal period after the growth hormone administration, the body weight decreased to almost its initial value. When the withdrawal period was increased, the body weight remained almost constant (Fig. 2, Table 1). The body weight of the controls showed only minor changes during the experimental period.
Table 1 (cont.)
25 μg \times 10 + 10, or 25 μg \times 10 + 15). Determination of accumulated growth in length, size of degenerative cell and calculation of accumulated cell production of proximal tibia. Width of undifferentiated zone, columnar zone and total width of proximal tibial growth plate. Weight of body and heart.

<table>
<thead>
<tr>
<th>Cartilage width</th>
<th>Weight</th>
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<tbody>
<tr>
<td>Undiff. zone (μm)</td>
<td>Columnar zone (μm)</td>
</tr>
<tr>
<td>12 ± 1</td>
<td>167 ± 13</td>
</tr>
<tr>
<td>12 ± 6</td>
<td>168 ± 24</td>
</tr>
<tr>
<td>I(-)</td>
<td>II(-)</td>
</tr>
<tr>
<td>9 ± 5</td>
<td>166 ± 13</td>
</tr>
<tr>
<td>II(-)</td>
<td>II(-)</td>
</tr>
<tr>
<td>4 ± 4</td>
<td>156 ± 17</td>
</tr>
<tr>
<td>III(-)</td>
<td>III(-)</td>
</tr>
<tr>
<td>9 ± 6</td>
<td>211 ± 18</td>
</tr>
<tr>
<td>I(-), II(-)</td>
<td>I**, II*</td>
</tr>
<tr>
<td>5 ± 2</td>
<td>193 ± 7</td>
</tr>
<tr>
<td>V(-), II*</td>
<td>V(-), II(-)</td>
</tr>
<tr>
<td>10 ± 4</td>
<td>187 ± 18</td>
</tr>
<tr>
<td>VI(-), III(-)</td>
<td>VI(-), III*</td>
</tr>
<tr>
<td>5 ± 2</td>
<td>158 ± 12</td>
</tr>
<tr>
<td>10 ± 2</td>
<td>148 ± 8</td>
</tr>
<tr>
<td>VIII*, IV(-)</td>
<td>VIII(-), IV(-)</td>
</tr>
</tbody>
</table>

Group-numerals below the values indicate result of statistical analysis (Student's t-test).
*** \( P < 0.001 \) ** 0.001 < \( P < 0.01 \) * 0.01 < \( P < 0.05 \) (-) \( P > 0.05 \).

Heart weight. – The weight of the heart ventricles showed the same changes as described for body weight (Fig. 2, Table 1). The ratio heart weight/body weight was almost constant for the various types of administration of growth hormone (Table 1), which agrees with earlier investigations (Tipton & Tcheng 1971; Thorngren et al. 1973b).
**DISCUSSION**

After hypophysectomy in the rat, the longitudinal bone growth gradually decreases and reaches a low basal growth rate, which depends on the age at hypophysectomy (Thorngren et al. 1973a). Female rats hypophysectomized at the age of 60 days have a very low basal growth rate after a post-operative period of 15 days (Thorngren et al. 1973b), i.e. when the growth hormone administration started in the present investigation. The cessation of the hormone administration in hypophysectomized rats in the present investigation is almost comparable to the withdrawal of endogenous growth hormone production after hypophysectomy.

During the administration and the following withdrawal period, there were different changes in the various growth parameters studied in the present investigation. For a review of the principles concerning endochondral bone growth, see Sissons (1956, 1971), Hansson (1967) and Ham (1969).
After 5 days administration of growth hormone, the width of the growth plate and the size of the degenerative cell had increased to their highest value (Figs. 1 and 2). A minor increase in the longitudinal bone growth and the calculated cell production was found, whereas the weight of the body and heart was unchanged (Figs. 1 and 2). The increase in the width of the growth plate depends on the increased cell proliferation and hypertrophy, whereas the resorption of the cartilage cells at the erosion line is still rather low.

After 10 days administration of growth hormone, the width of the growth plate had decreased somewhat, and the size of the degenerative cell was unchanged. The longitudinal bone growth and the calculated cell production, however, had increased further. The body and heart had increased in weight and reached their highest values (Figs. 1 and 2). The decrease in the width of the growth plate is due to the increased activity in the resorption process of the degenerative cells at the erosion line.

When growth hormone administration was given for 10 days followed by a 5-day period after withdrawal of administration, the width of the growth plate remained constant, and the size of the degenerative cells was almost unchanged. The longitudinal bone growth and the calculated cell production increased further, both processes having accelerated (Figs. 1 and 2). The weight of the body and heart had decreased to their initial values and then remained at a low level (Fig. 2). Thus, an equilibrium had been achieved between the cell production in the growth plate and the cell destruction at the erosion line in the process of bone formation.

When the period after cessation of the growth hormone administration was increased to 10 days, the width of the growth plate decreased and reached the same value as the controls. The size of the degenerative cell decreased somewhat further. The longitudinal bone growth and the calculated cell production showed a constant value as compared to the 5-day withdrawal period indicating that the bone growth had ceased. The decrease in the width of the growth plate and in the size of the degenerative cell reflects the cessation of cell proliferation along with the decreasing resorption process.

With the 15-day withdrawal period, the effect was the same as for the 10-day withdrawal period.

Thus, the administration of growth hormone initiates a growth process in the different growth parameters studied. The time necessary to reach an optimum growth effect was, however, found to differ for the growth parameters. After withdrawal of the growth hormone administration, the effect on the growth parameters studied also differed.

All the growth processes studied had stopped when the 10-day administration period of growth hormone was followed by a withdrawal period of 10 days. The growth effect of the given dose had then ceased. Only the size of the degenerative cell was still greater than in the controls (Figs. 1 and 2). The
degenerative cells determined at this time are the result of an earlier proliferation process initiated by growth hormone. If the withdrawal period had been longer, the cells would probably have reached the same value as for the controls.

The time necessary to achieve maximum accumulated growth in length after the withdrawal of growth hormone administration may depend on the dose given and the period of administration. The effect of the endogenous production of growth hormone by the pituitary is much higher than the dose given in the present investigation, which in a previous investigation (Thorngren et al. 1973b) has shown a significant growth promoting effect in hypophysectomized rats. As shown in the present investigation, the effect of the given dose on the longitudinal bone growth terminated within a 5-day period after the withdrawal of growth hormone administration. Previously, it was found that untreated rats had a low basal growth rate within 10 days after hypophysectomy (Thorngren et al. 1973a). Thus, a 10-day period after the withdrawal of the growth hormone administration seemed to be sufficient to obtain a maximum effect on the accumulated growth in length of any administered dose of growth hormone.

The rate of longitudinal bone growth is equal to the product of two factors in the growth plate: the rate of production of new cells per column and the size of the degenerative cell (Sissons 1955; Walker & Kember 1972; Thorngren & Hansson, in press). The changes in the size of the degenerative cell found in the present investigation were minor. Thus, the changes in longitudinal bone growth found were predominantly due to changes in the accumulated cell production (Fig. 1). This agrees with earlier investigations (Thorngren & Hansson, in press). The small but insignificant variation in the degenerative cell size during the growth hormone administration, and following the withdrawal periods relevant to this investigation, indicates that any of the values of the degenerative cell size can be used for the calculation of cell production.

It has been claimed that the rate of longitudinal bone growth is directly proportional to the width of the growth plate (Rang 1969). In the present investigation, this relation was not found, nor was it found under experimental conditions in earlier investigations (Sissons 1956; Kember 1972; Thorngren & Hansson 1973a and in press). In normal animals, however, there is a relation between the width of the growth plate and the rate of bone growth (Thorngren & Hansson, in press).

The continued growth effect found in the present investigation after withdrawal of growth hormone administration may be caused by a depot effect of growth hormone and a delayed effect in the endochondral growth process.

In the present investigation, a depot effect of the administered growth hormone might have produced the continued longitudinal bone growth found after withdrawal of growth hormone administration. However, the injected volumes

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are small (0.1 ml) and would be resorbed rather quickly, so this effect is probably very small, since immunological determination of im injected human growth hormone in man has shown a peak in the serum concentration at about 3 h after the injection and undetectable concentration at about 24 h after the administration (Frasier et al. 1969). Furthermore, the half time for growth hormone in plasma is known to be about 30 min when determined immunologically (Glick et al. 1964).

The delayed effect in the endochondral growth process seems most probable. In this process, the cartilage cells are stimulated to mitotic division by growth hormone, probably indirectly through the production of somatomedin (Rigal 1964; Kember 1971; Daughaday et al. 1972). As shown with sulphate incorporation studies in vivo in hypophysectomized mice given ip injection of ovine growth hormone, there is a time-lag in the sulphation activity after a single dose, and the effect is maintained in vivo for at least 38 h after a single dose of growth hormone (Herbai 1971). The main cause of the latency observed may be due to the time needed for growth hormone to initiate somatomedin (sulphation factor) synthesis.

Clinically, the effects of growth hormone in hypopituitary patients is known to last for several days after the last injection (Beck et al. 1957; Korner et al. 1959). Experimentally, in most investigations (Asling & Evans 1956; Evans et al. 1966) on the growth stimulation by growth hormone in hypophysectomized rats, the hormone administration has been continued until the end of the experimental period, after which the animals have been killed. In the tibia test (Greenspan et al. 1949) where the growth hormone-dependent increase in the width of the cartilage growth plate is used as bioassay for growth hormone, the growth plate decreases in width again after the withdrawal of growth hormone administration. A continued growth effect after withdrawal of the growth hormone administration, as shown in the present investigation, has not previously been observed.

The withdrawal effect found in the present investigation, with tetracycline as intravital marker of the longitudinal bone growth, can be used to increase the growth response in the bioassay of growth hormone. By this means the full effect of a dose of growth hormone is recorded, and the total number of cells produced by the dose can be calculated.

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