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Evidence suggesting that the direct growth-promoting effect of growth hormone on cartilage *in vivo* is mediated by local production of somatomedin

(insulin-like growth factor I/hypophysectomized rat/long bone growth)

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ABSTRACT **We have used a catheterization system that permits chronic infusion into the arterial supply of one hindlimb of rats to study the direct effects of rat growth hormone and human somatomedin C on growth of the tibial epiphyseal cartilage plate in hypophysectomized rats. Rat growth hormone (0.4 μg per day) or human somatomedin C (0.25, 1, or 4 μg per day) stimulated growth of the epiphyseal plate of the infused limb but not of that of the contralateral noninfused limb. The somatomedin C had a dose-related effect. Rabbit antiserum to human somatomedin C, but not normal rabbit serum, completely abolished the direct growth effect of the rat growth hormone when it was co-infused with the hormone. These results support the concept that growth hormone stimulates long bone growth by inducing local production of somatomedin, which in turn stimulates cell proliferation in an autocrine or paracrine fashion. However, they do not exclude the possibility that serum somatomedin may also play a role.**

The somatomedin hypothesis of Salmon and Daughaday (1) maintains that the growth-promoting actions of growth hormone (GH) are indirect and act via GH-dependent plasma factors named somatomedins or insulin-like growth factors. This hypothesis has evolved to include the concept that somatomedins are produced primarily by the liver in response to GH (2–4). Although most of the early evidence in support of this theory was derived from *in vitro* work, Schoenle *et al.* (5) recently demonstrated a dose-related growth-promoting effect of systemically infused human somatomedin (hSm) on the proximal tibial epiphyseal plate of hypophysectomized rats. However, a local effect of GH on tibial cartilage growth has also been demonstrated in hypophysectomized rats *in vivo* by direct injection into the proximal tibial epiphyseal plate (6, 7), by intraarticular injection into the knee joint or administration into the epiphysis by implanted cannula (8), and by chronic infusion into the arterial supply of one hindlimb (9). In addition, numerous cells and tissues have been shown to produce somatomedins *in vitro*, either spontaneously or in response to GH (10–18). Thus, it is possible that GH exerts its direct effects by stimulating local production of somatomedins.

To investigate this possibility we used the system of Schlechter *et al.* (9), which permits chronic infusion into the arterial supply of one hindlimb of a rat. With this procedure, we have demonstrated a direct growth-promoting effect of human somatomedin C (hSm-C) on the proximal tibial epiphyseal plate of hypophysectomized rats. In addition, we have found that the local effect of rat GH (rGH) could be completely abolished by co-infusing an antiserum raised against hSm-C.

MATERIALS AND METHODS

Male Long–Evans rats (125–150 g) were obtained from our breeding colony. Husbandry conditions were as described (9). The animals were hypophysectomized by the transauricular approach (19) 14 days prior to catheterization. On the day of catheterization, they were reanesthetized (9) and the right superior vesical artery, which is a small medial branch of the right common iliac artery, was isolated and clamped at a point distal to the common iliac artery. The infusion system and catheterization technique used in these experiments have been described in detail (9). Briefly, a polyethylene catheter was inserted into a hole made in the superior vesical artery and advanced until the tip just abutted the junction with the common iliac artery. The catheter was subsequently tied in this position with a ligature. The catheter and connected Alzet osmotic minipump (2001; Alza, Palo Alto, CA) were then placed in the abdominal cavity, and the muscle wall and skin were separately sutured closed.

The hormones (rGH, NIH B-9, 0.4 μg per day; hSm-C, prepared by E.M.S., 0.25, 1.0, and 4.0 μg per day) were dissolved in a solvent consisting of 1.6% glycerol/0.02% sodium azide in double-distilled water and adjusted to pH 8.5 (9). Ten percent of the pump volume was a sodium heparin solution (1000 USP units/ml; Lypho Med, Chicago, IL).

In some animals, the GH was co-infused with either normal rabbit serum or rabbit antiserum to hSm-C, which was produced by direct intranodal injection of hSm-C (20 μg) as described by Sigel *et al.* (20). Booster injections (10 μg) were administered twice at 2-month intervals. At a final dilution of 1:16,000, the antiserum bound 30% of labeled hSm-C which had a specific activity of 150 μCi/μg (1 Ci = 37 GBq). The antiserum had 3% crossreactivity with insulin-like growth factor II, and did not crossreact with insulin, GH, prolactin, bradykinin, placental lactogen, or thyroid-stimulating hormone.

For the experiments with infusion of serum, the solvent was prepared at 10 times normal concentration, and the pump was filled with 80% serum, 10% heparin, and 10% of the concentrated solvent with dissolved hormone.

Seven days after the catheterization, the animals were killed by injection of an overdose of sodium pentobarbital. The sellar region was then examined for the presence of pituitary remnants, the pump and catheter were examined for patency, and the tibiae were removed and processed for measurement of epiphyseal plate width. Only animals with no

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Abbreviations: hSm-C, human somatomedin C; rGH, rat growth hormone.

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pituitary remnants, significant testicular regression, and weight loss of at least 1 g/day were included. These criteria have previously been shown in our laboratory to indicate lack of radioimmunoassay-detectable GH in tissue scrapings from the sellar region or in serum samples of hypophysectomized male rats (S.M.R., unpublished observations).

Measurement of the epiphyseal plate width was performed as described (9). The difference in the mean epiphyseal plate widths of each infused vs. noninfused leg was calculated, and the significance was determined by the Student's *t* test for paired observations. It has been shown previously that the width of this plate is highly correlated with the rate of long bone growth (21).

RESULTS

The mean epiphyseal plate widths of both infused and noninfused limbs for all groups are presented in Figs. 1 and 2. Infusion of the solvent into one hindlimb did not affect the width of the epiphyseal plate of the infused limb when compared with the noninfused limb. Also, in no case did the mean plate width of the noninfused limbs of the various treatment groups differ from that of the solvent-infused group. Thus, none of the treatments had a systemic effect.

Infusion of hSm-C at 0.25, 1, or 4 μg per day each caused a significant unilateral increase in epiphyseal plate width compared to solvent-infused animals ($P < 0.001$). The mean differences in widths were 9.4 ± 0.7 μm, 18.2 ± 1.4 μm, and 24.0 ± 4.5 μm for the three doses of somatomedin, respectively, and 3.2 ± 1.2 μm for the solvent (Fig. 1). The responses to the intermediate and high doses (1 and 4 μg per day) are significantly greater than that to the lower one ($P < 0.001$), but the responses to the intermediate and high doses were not significantly different from each other.

Infusion of rGH in solvent or in normal rabbit serum caused comparable degrees of unilateral growth of the epiphyseal plate (Fig. 2). The mean differences in plate widths were 23.1 ± 4.6 μm and 23.2 ± 2.8 μm, respectively, which are both significantly different from that of the solvent infused animals ($P < 0.001$). However, when the GH was co-infused with the antiserum to hSm-C, the growth-promoting effect of the hormone was completely nullified, resulting in a mean difference of −2.7 ± 3.4 μm.

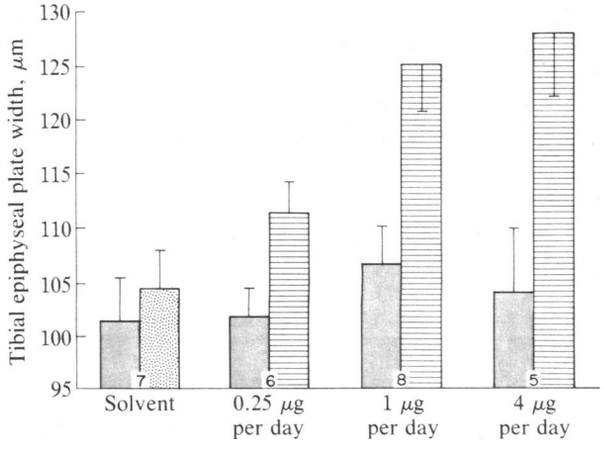

FIG. 1. Effects on tibial epiphyseal plate width of chronic infusion via a minipump of solvent or of hSm-C (0.25, 1, or 4 μg per day) for 7 days into the arterial supply of one hindlimb of male rats 14 days after hypophysectomy. The number of animals per group is indicated at the bottom of the bars. Solid bars represent the width of the noninfused epiphyseal plates of that group.

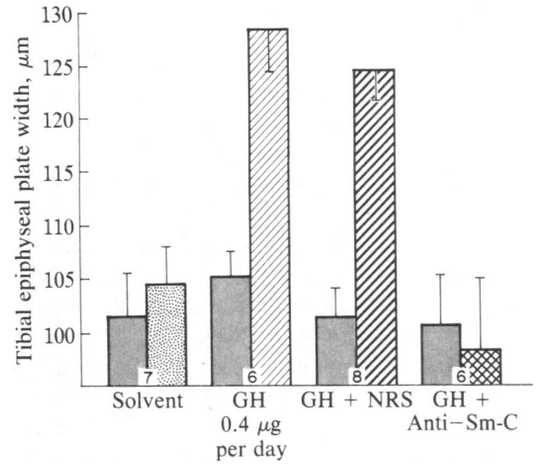

FIG. 2. Effects on tibial epiphyseal plate width of chronic infusion of solvent or rat GH (0.4 μg per day) alone or with normal rabbit serum (NRS; 80% of pump volume) or rabbit antiserum to hSm-C (Anti-Sm-C; 80% of pump volume) into the arterial supply of one hindlimb of male hypophysectomized rats. Solvent data are the same as in Fig. 1. Other details are given in the legend to Fig. 1.

DISCUSSION

Previous work has demonstrated that GH can act locally *in vivo* in a dose-related manner to promote long bone growth when administered by direct injection (6–8) or by local arterial infusion (9). In the present study, we have confirmed the local growth-promoting action of GH, and we have demonstrated such an action of human Sm-C when it is infused into the arterial supply of one hindlimb. In addition, we have shown that the response to Sm-C is dose related. It seems probable that the two higher doses (1 and 4 μg per day) approached a maximal growth-promoting effect of Sm-C in this system, as the mean epiphyseal plate widths of the animals receiving these treatments were not significantly different from each other. Russell and Spenser (7) demonstrated a growth-promoting effect of Sm-C when it was administered to animals 8 days after pituitary ablation, but not when administered after 14 days. In addition, they were unable to obtain a dose-related effect in their animals. This discrepancy between our results and those of Russell and Spencer (7) may be due to differences in the mode of Sm-C administration. They used daily bolus intraepiphyseal plate injections for 4 days, in contrast to the chronic intraarterial infusion for 7 days used in our present study. The insulin-like growth factor preparations used in these two studies were obtained from the same source (E.M.S.; ref. 22). In addition, Isgaard *et al.* (8) have reported a small local effect of bacterially derived Sm-C (5 μg per day) when administered daily for 5 days via an implanted cannula in the epiphysis of one hindlimb of rats 14 days after hypophysectomy.

The absence of a response to rGH when it was infused in the presence of antiserum to hSm-C, while it was effective when infused in the presence of normal rabbit serum, provides cogent evidence that the direct action of GH is mediated by locally produced somatomedins. Other evidence is consistent with this suggestion. A growth-promoting action of GH *in vitro* has been reported in both murine (23) and human (24) erythroid precursor cells and, more pertinent, in cultured chondrocytes from rabbit ear and rat rib growth cartilage (25). In addition, GH potentiates the formation of large-sized colonies in suspension cultures of rat epiphyseal plate chondrocytes (26), and labeled human GH binds specifically to rabbit cartilage cells (27, 28) and to cultured human fibroblasts (29). These growth-promoting effects of GH *in vitro* may be mediated by local production of

somatomedins because embryonal rat tibiae (10), human fibroblasts (11–13), and multiple rat tissues (14) produce Sm-C in response to GH in culture. Although the original proposal for a mediator of GH action was based on the fact that GH had small and inconsistent growth effects *in vitro* (1), more recent work has shown convincing growth effects; the discrepancies between the earlier work (1) and the more recent studies (23–25) could be a consequence of variations in cell populations or methodologies used.

While the liver is generally accepted as the primary source of somatomedins (2–4), a number of nonhepatic tissues and cells, both pre- and postnatally, have been reported to produce these factors. For example, Sm-C is produced in culture by a number of fetal mouse tissues (15), by neonatal rat islet cells (16), and by porcine smooth muscle cells (11, 17) and other tissues (10–14), as mentioned above. Significantly, it has been demonstrated that the somatomedin produced by human fibroblasts and porcine smooth muscle cells in culture has a functional role in the regulation of DNA synthesis in those cells (11). With regard to our results, it is significant that Andersson and coworkers (18) found Sm-C by an immunohistochemical method in chondrocytes of epiphyseal growth plates, in joint cartilage, and in osteoblasts and odontoblasts, as well as a number of other rat tissues. Although these various sources of somatomedins may not contribute significantly to serum Sm-C levels, it is possible that each GH target tissue can produce sufficient quantities of the growth factor to act in an autocrine and/or paracrine manner, as proposed recently by a number of workers (13, 17, 26, 30–32). Green and coworkers (30) have proposed that GH plays a dual effector role in promoting tissue growth. Based on their work with adipocyte conversion of 3T3 cells (33–35), these investigators suggested that GH stimulates differentiation of prechondrocytes, and that somatomedin acts as a mitogen only on the differentiated cells. Although Green *et al.* (30) did not stipulate that the growth factors may be produced by the differentiated cells themselves, Isaksson *et al.* (26, 31) suggested that during the differentiation process, GH may induce the expression of genes for local growth factors in chondrocytes.

In conclusion, our results support the primary component of the somatomedin theory (i.e., that somatomedins and insulin-like growth factors mediate the growth-promoting action of GH *in vivo*). However, our data indicate that local somatomedin production may be more important for promoting growth *in vivo* than was previously realized. Local somatomedin production in response to GH could explain some clinical inconsistencies, such as the report that a greater dose of GH is required to achieve normal serum somatomedin levels than to achieve a normal rate of growth (36), and the lack of correlation between serum somatomedin levels and growth rate in some cases in humans (37, 38) and in rats (39). However, the relative contribution of locally produced vs. hepatically derived somatomedins in mediating the growth effects of GH is unknown at this juncture.

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