Changes in tissue levels of growth hormone, insulin-like growth factor-I, and somatostatin in the femurs of hind-limb immobilized rats

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Submitted 00-03-28. Accepted 00-10-20

ABSTRACT – Immobilization of an extremity causes skeletal muscle atrophy and a dramatic increase in bone resorption. Growth hormone (GH) is known to play an important role in bone remodeling mediated in part by local insulin-like growth factor-I (IGF-I). In this study, we investigated changes in the levels of GH and IGF-I peptide in bone extracts from the femur after hind-limb immobilization for 5 days, 2, 4, and 8 weeks. The levels of somatostatin, which interacts with GH, were also measured in the bone extracts. GH levels increased after 8 weeks of hind-limb immobilization whereas the IGF-I concentrations increased after 2 weeks, but returned to control levels at 4 weeks, and decreased after 8 weeks of immobilization. The somatostatin levels in the bone extracts increased only after 8 weeks of hind-limb immobilization. Our findings suggest that, after hind-limb immobilization, changes in the concentrations of GH, IGF-I, and somatostatin in bone may mediate bone resorption either directly or through interaction with other factors.

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The balance between bone resorption and bone formation depends on availability of a series of systemic and local hormones and growth factors (Raisz 1988). Immobilization-induced osteoporosis is caused by increased bone resorption and decreased formation (Yeh et al. 1993, Zeng et al. 1996), but the main feature during immobilization is a fast increase in bone resorption (Lueken et al. 1993). GH and IGF-I are important for normal postnatal longitudinal bone growth (Bouillon 1991). However, these peptides affect osteoblasts and osteoclasts, which remain important even after the closure of epiphyseal growth plates (Raisz 1988, Bouillon 1991). In experimental studies, injection of GH directly into the growth plate of rat tibia stimulated longitudinal bone growth at the site of injection (Aspenberg et al. 1985). In the diaphysis, GH treatment stimulated cortical bone formation and mechanical strength by increasing subperiosteal bone formation (Isaksson et al. 1987, Andreassen et al. 1995). GH treatment stimulated fracture healing and increased mechanical strength in rat tibial fractures (Bak et al. 1991).

IGF-I is secreted by bone cells where it promotes their proliferation and differentiation in an autocrine/paracrine fashion (Wergedal et al. 1990, Baylink et al. 1993). IGFs are the most abundant growth factors produced by bone cells and stored in bone. IGF-I has been shown to increase collagen synthesis, matrix apposition and cell replication in the osteoprogenitor region and in the zone of mature osteoblasts (Hock et al. 1988). Because of its potent mitogenic propensity for osteoblasts, IGF-I has been thought to be able to act as a formation-stimulating drug in the treatment of age-related osteoporosis (Canalis 1994). Besides IGFs, the bone matrix in rats contains other growth factors such as transforming growth factor-beta, fibroblast growth factor, platelet-derived growth factor, and bone morphogenetic proteins (Baylink et al. 1993).

Somatostatin and its analogs inhibit the release of GH and the secretion of growth factors such as IGF-I (Schally 1988). This action was noted in patients who received a somatostatin analog for the

treatment of tumors potentially dependent on IGF-I (Pollak et al. 1989).

In the present study, we used a rat hind-limb immobilization model to investigate the effects of disuse on GH, IGF-I, and somatostatin concentrations in tissue extracts of rat femora at different times: 5 days, 2, 4, and 8 weeks after hind-limb immobilization.

Animals and methods

Animals and treatment

6-month-old male Sprague-Dawley rats (n 40) were used. They were kept in separate cages and fed food and water ad libitum. They were randomly divided into immobilization (n 32) and control (n 8) groups. In the immobilization group, the right hind limb of the rats was immobilized against the abdomen by an elastic adhesive bandage, as described by Lindgren (1976), and they were randomly divided into 4 groups: 5 days, 2 weeks, 4 weeks, and 8 weeks (8 rats in each group). The bandages were made and checked as described previously (Suliman et al. 1997). The rats were killed by decapitation under fentanyl-fluanisone anesthesia, 0.4 mL/kg i.m. The right femur was dissected from each rat, and the soft tissues and bone marrow were removed. The bones were weighed and immediately frozen in isopentane precooled in liquid nitrogen (–150 °C) and stored at –70 °C, pending use.

For extraction, the bones were cut and crushed into small fragments. The samples were then boiled for 10 min in 10 volumes of 2 M acetic acid in 4% EDTA and homogenized (1 min) with a polytron, sonicated for 20 seconds, and centrifuged for 20 min at 13,000g. The supernatant was lyophilized and stored at –20 °C, pending use.

GH analysis was performed, using a rat radioimmunoassay (RIA) kit (Amersham Life Science, Little Chalfont Buckinghamshire, U.K.), as described by the manufacturer.

For IGF-I determination with RIA, we used a rat IGF-I RIA kit (Diagnostic Systems Laboratories, Texas, USA). The procedure follows the basic principles of RIA but, in addition, there is an acid-ethanol extraction procedure which precedes the assay. In the acid extraction procedure, 50 μL of the reconstituted sample and 200 μL of extraction solution (ethanol HCl) were added together, mixed, and incubated for 30 min at room temperature. The mixture was then centrifuged at 3500 g for 3 min at room temperature. From the clear supernatants, 100 μL was transferred into another set of tubes and into each we added 500 μL of neutralizing solution (sodium azide buffer) was added. From the neutralized sample extracts, 100 μL samples were used in duplicate for the RIA assay, according to the instructions of the manufacturer.

Somatostatin concentration in the femur extracts was determined using a rat somatostatin RIA kit (Peninsula Laboratories Europe Ltd., St. Helena, U.K.), as described by the manufacturer.

The experiment was approved by the Ethics Committee of our Institute.

Statistics

Statistical analysis was performed, using Fisher's least-significant difference method for multiple comparisons in a one-way analysis of variance (ANOVA). Significance was considered as $p<0.05$.

Results

GH levels in the tissue extracts from the immobilized group were higher ($p = 0.002$) in the 8-week group than in the bone extracts from the control group. No significant differences were observed in the 5 day, 2-, and 4-week groups when compared to the control group (Figure 1).

The IGF-I levels in the femur were not significantly higher than in the controls at 5 days and peaked at 2 weeks when they were significantly ($p= 0.04$) higher . They then decreased to the control level at 4 weeks and finally became significantly ($p = 0.002$) lower than normal at 8 weeks (Figure 2).

Somatostatin levels in the tissue extracts of the immobilized femora showed no significant differences after 5 days, 2 weeks, and 4 weeks of immobilization, as compared to the controls. After 8 weeks of immobilization, the somatostatin concentrations were significantly ($p = 0.04$) higher in the bone extracts of the immobilized rat femora than in the controls (Figure 3).

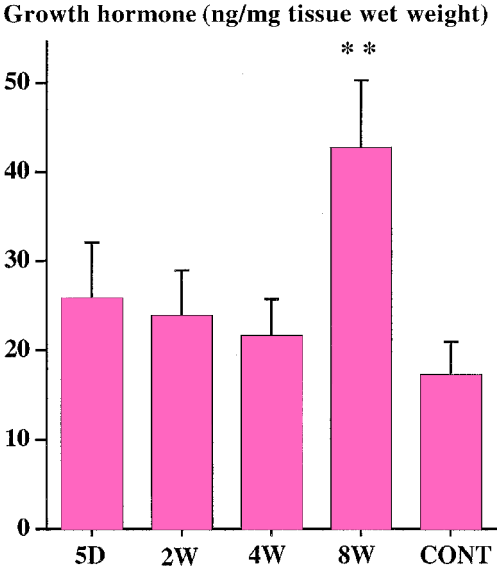

Figure 1. GH levels obtained by RIA in extracts of bone (femoral) tissue (ng/mL), as described in Animals and methods. Each column corresponds to mean ± SEM (n 8). $P < 0.002$, compared to the control.

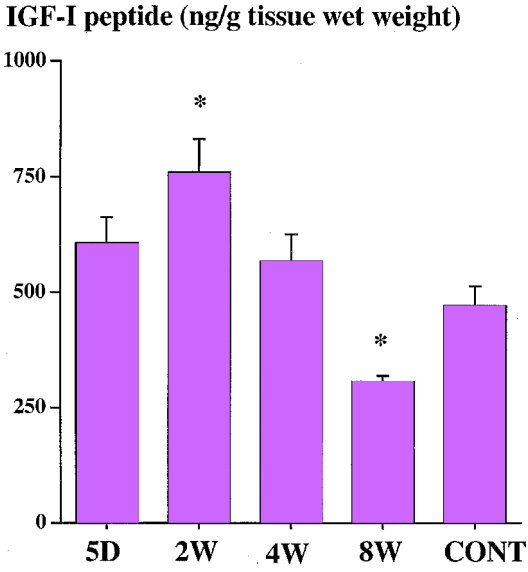

Figure 2. IGF-1 levels obtained by RIA in extracts of bone (femoral) tissue (ng/mL), as described in Animals and methods. Each column corresponds to mean ± SEM (n 8). $P < 0.04$, $p < 0.002$, compared to the control.

Discussion

In the literature, several in vivo and in vitro effects of GH and IGF-I have been reported. GH increases the number of osteoclasts in the metaphyseal bone of the proximal tibia of hypophysectomized rats (Lewinson et al. 1993). Stracke et al. (1984) found that GH increased alkaline phosphatase activity in the culture medium from embryonal rat tibias in tissue culture. GH stimulates the proliferation of osteoblasts and, in some studies, it stimulated functional differentiation of bone cells (Ernst and Froesch 1988, Morel et al. 1993). Moreover, addition of GH to cultured tibiae led to an increase in IGF-I in the culture medium, indicating that GH stimulates IGF-I production in the bone specimen (Stracke et al. 1984). GH stimulates not only osteoblasts, but also osteoclasts (Nishiyama et al. 1996). However, the mechanism of this effect is unclear.

In our study, we observed a significant increase in the GH and somatostatin concentrations in the bone extracts of the immobilized rat femora. The exact cause of the increase in GH is not known, but it is unlikely to be a stress-induced increase in the pituitary production because immobilization-induced stress in the rat is known to reduce GH levels (Armario et al. 1993) and increased level of somatostatin levels (Benyassi et al. 1993).The increase in tissue concentration of GH may be due to an increase in its receptors and/or their binding affinity acting to increase its local availability. The proposed switch in GH effects from bone formation to resorption may have been caused by unloading which reduces the strain on hind limb bones to a level below the threshold required to stimulate formation (Frost 1997).

On the other hand, IGF-I inhibits GH and down-regulates its receptors (Slootweg et al. 1995). Thus the increase in GH levels in the bone extracts may be due to lack of inhibition from IGF-I which is significantly decreased at 8 weeks. In tissue cultures, IGF-I is dependent on GH and mediates its function. This has been shown in bone cell cultures where endogenous IGF-I was sequestered by an antiserum to IGF-I. As a result, the proliferative action of GH was abolished (Ernst and Froesch 1988), indicating that local IGF-I is important for GH-induced cell proliferation. Circulating levels of IGF-I are GH-dependent, but the local production of IGF-I in the periphery is GH-independent (Dore et al. 1995). In cell cultures, IGF-I is

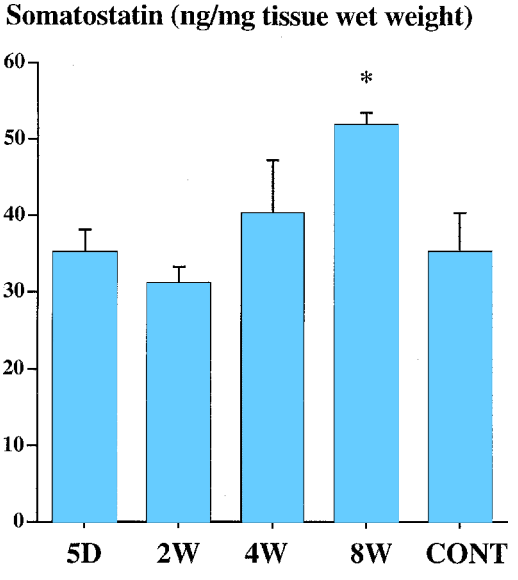

Figure 3. Somatostatin levels obtained by RIA in extracts of bone (femoral) tissue (ng/mL), as described in Animals and methods. Each column corresponds to mean ± SEM (n 8). $P < 0.04$, compared to the control.

locally regulated by PTH, estrogen, and cortisol, as well as a variety of local growth factors (Canalis 1994, Swolin et al. 1996). Other factors which are important for the action of IGF-I are IGF-binding proteins (IGFBPs), especially IGFBP-3, -4 and -5 for the bioactivity of IGF-I in bone tissue (Hayden et al. 1995). Bone cells synthesize the 6 reported IGFBPs, and these binding proteins could play a major role in the deposition of IGF-I in bone matrix. IGF-I has potent stimulatory effects on osteoblasts, as shown by increased osteocalcin and type I collagen synthesis, which indicates increased bone matrix apposition (Canalis et al. 1993). It also inhibits the expression of interstitial collagenase, which reduces collagen degradation and preservation of bone matrix.

In vivo administration of IGF-I increases biochemical markers of bone formation and bone resorption (Johansson et al. 1992). The factors determining the regulation of bone IGF-I in vivo, however, are complex and they may be modified by activity. It has been shown that a reduction in serum IGF-I in aging people correlates with a decline in physical fitness (Kelly et al. 1990). In a recent study, we found a significant reduction in skeletal muscle IGF-I after hind-limb immobilization in the rat (Suliman et al. 1999). These observations are in line with animal studies that showed activation of the IGF-I and -II genes in experimental muscular hypertrophy (DeVol et al. 1990). This would suggest a correlation between changes in IGF-I synthesis and muscle mass, and similar mechanisms may occur in bone (Canalis 1994).

IGFs are the most abundant growth factors produced by bone cells and stored in the bone matrix (Mohan and Baylink 1991). The relative distribution of IGF-I and -II differ between rodents and humans. The rodent bone extracts contain more IGF-I than IGF-II, but the reverse is true in humans (Mohan and Baylink 1991). In our study, IGF-I in the bone extracts increased significantly after 2 weeks, returning to control levels after 4 weeks, and finally decreased significantly after 8 weeks of immobilization. The local increase in IGF-I concentrations seen after 2 weeks of immobilization may in part come from stored IGF-I released from the bone matrix. The released IGF-I may have acted in an autocrine/paracrine manner and stimulated the production of IGF-I from bone cells (Baylink 1993). These results are in line with a previous report by Bikle et al. (1995), in which hind-limb unloading resulted in a significant increase in IGF-I mRNA and peptide. The tissue levels of IGF-I returned to normal after 4 weeks of immobilization most probably because of a reduced release of the stored IGF-I in response to declining bone resorption. After 8 weeks of immobilization, when the osteoporotic stage can be expected to be well-established (Suliman et al. 1997), a significant reduction in the concentrations of IGF-I in bone extracts was noted.

Somatostatin is regarded as a GH release inhibitor. We have recently reported the presence of somatostatin in mature bone matrix and bone marrow (Elhassan et al. 1998). The increased concentrations of somatostatin after 8 weeks of immobilization may be related to an increased concentration of GH. Further studies should deal with the role of somatostatin in bone and its interaction with IGF-I and GH in bone formation and resorption. It should be mentioned, however, that systemic somatostatin reduced serum IGF-I levels in patients with neoplasms possibly dependent on IGF-I (Pollak et al 1989).

Our data indicate that GH and IGF-I may not be only associated with bone formation but that elevated levels of these peptides may occur in conditions of increased net bone resorption, as after immobilization. The findings may indicate that mechanical factors can influence their effects either directly or through the release of other local factors. We think that mechanical stress has profound effects on bone tissue regulation and that its coupling to chemical mediators should be studied further.

This project was supported by grants from the AO Foundation, Switzerland, the Swedish Medical Research Council, and KI Funds.

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