Vibration-induced activation of muscle afferents modulates bioassayable growth hormone release

1Department of Physiological Science, 2Brain Research Institute and 3Department of Neurobiology, University of California, Los Angeles 90095; and 4Life Science Division, National Aeronautics and Space Administration-Ames Research Center, Moffett Field, California 94035

Submitted 13 August 2003; accepted in final form 30 January 2004

Gosselink, K. L., R. R. Roy, H. Zhong, R. E. Grindeland, A. J. Bigbee, and V. R. Edgerton. Vibration-induced activation of muscle afferents modulates bioassayable growth hormone release. J Appl Physiol 96: 2097–2102, 2004. First published February 6, 2004; 10.1152/japplphysiol.00855.2003.—The effects of tendon vibration on bioassayable growth hormone (BGH) secretion from the pituitary gland were investigated in anesthetized adult male rats. The tendons from predominantly fast-twitch ankle extensor muscles (gastrocnemius and plantaris) or a predominantly slow-twitch ankle extensor (soleus) were vibrated by using a paradigm that selectively activates group Ia afferent fibers from muscle spindles. The lower hindlimb was secured with the muscles near physiological length, and the tendons were vibrated for 15 min at 150 Hz and a displacement of 1 mm. Control rats were prepared similarly, but the tendons were not vibrated. Compared with control, vibration of the tendons of the fast ankle extensors markedly increased (160%), whereas vibration of the slow soleus decreased (68%), BGH secretion. Complete denervation of the hindlimb had no independent effects on the normal resting levels of BGH, but it prevented the effects of tendon vibration on BGH secretion. The results are consistent with previous findings showing modulation of BGH release in response to in vivo activation or in situ electrical stimulation of muscle afferents (Bigbee AJ, Gosselink KL, Grindeland RE, Roy RR, Zhong H, and Edgerton VR. J Appl Physiol 89: 2174–2178, 2000; Gosselink KL, Grindeland RE, Roy RR, Zhong H, Bigbee AJ, and Edgerton VR. J Appl Physiol 88: 142–148, 2000; Gosselink KL, Grindeland RE, Roy RR, Zhong H, Bigbee AJ, Grossman EJ, and Edgerton VR. J Appl Physiol 84: 1425–1430, 1998). These data provide evidence that this previously described muscle afferent-pituitary axis is neurally mediated via group I afferents from peripheral skeletal muscle. Furthermore, these data show that activation of this group Ia afferent pathway from fast muscles enhances, whereas the same sensory afferent input from a slow muscle depresses, BGH release.

pitiutary; proprioception; tendon vibration; muscle afferent-pituitary axis

Numerous studies have demonstrated that the pituitary gland releases a biologically active hormone or growth factor that stimulates bone growth yet is distinct from the classic growth hormone (GH) (4, 13, 18–23, 27–30, 33). Our laboratory and others have referred to this factor as bioassayable GH (BGH), on the basis of the method for measuring its activity (20), which contrasts with the fact that GH and its variants are most commonly measured by immunoassay or other immunofunctional techniques (1, 2, 13). Although both GH and BGH are of pituitary origin and stimulate bone growth in rats, numerous studies have demonstrated marked differences in the regulation of these two factors. First, metabolic stimuli such as fasting, cold exposure, or insulin-induced hypoglycemia induce differential responses of GH and BGH in rats (14). Second, neuromuscular activation can result in changes in BGH release with no effect on GH secretion (see below). Third, high- and low-density pituitary somatotrophs produce different GH-to-BGH ratios in terms of both their content and secretory profiles (23).

A number of previous studies have provided evidence that BGH release from the pituitary can be mediated via inputs from skeletal muscle afferents, with little or no effect on GH secretion. For example, BGH levels are modulated by exercise, chronic changes in the loading status of the musculoskeletal system, and the activation of afferent inputs from peripheral skeletal muscles. BGH secretion was elevated after 15 min of aerobic exercise (treadmill running) in rats (4) and by a brief, high-resistance exercise (unilateral plantar flexion) in humans (28). Chronic unloading of the musculature achieved by 17 days of spaceflight (29) or bed rest (28) eliminated the normal BGH response to exercise in humans, with recovery of the response occurring after 2–4 days of reambulation. In rats, hindlimb unloading (22) or spaceflight (21–23) depressed pituitary BGH release in both in vivo and in vitro experiments. Furthermore, our laboratory demonstrated that a single bout (15 min) of intermittent electrical stimulation of large-diameter, low-threshold fibers from predominantly fast-twitch ankle extensor muscles induced BGH release in anesthetized rats (19), whereas stimulation of these afferents from a predominantly slow-twitch ankle extensor inhibited BGH secretion (18). The pattern and strength of the electrical stimulus that modulated the BGH response in these experiments suggested that this effect was mediated by group I and/or II muscle afferents. In addition, McCall et al. (30) showed that vibration of the surface of the tibialis anterior in humans increased circulating BGH concentrations, whereas there was no significant effect after vibration of the soleus. The vibration stimulus has been shown previously to specifically excite muscle spindle afferents, with primary (group Ia) endings being preferentially activated over secondary (group II) endings (7, 10, 11, 26). All of these results are consistent with a muscle afferent-pituitary axis (27, 33) through which muscle activity can regulate the release of BGH, a factor known to be a positive effector of growth in the skeletal system (13, 20). Furthermore, there is a marked disruption of this axis after chronic periods of unloading such as occurs with spaceflight or long-term bed rest.
Importantly, GH secretion was not markedly or consistently altered by any of these experimental paradigms. The purpose of the present study was to more stringently test the hypothesis that group Ia afferents from muscle spindles can modulate BGH secretion. Using in situ tendon vibration, we show that selective activation of these afferents from predominantly fast ankle extensor muscles specifically induces BGH secretion to a level similar to that seen in rats after treadmill exercise or electrical nerve stimulation, whereas activation of slow ankle extensor Ia fibers inhibits BGH release. These data indicate that the muscle afferent-pituitary axis is driven to a great extent by inputs from the primary endings of muscle spindles. Portions of these data have been previously published in abstract form (17).

METHODS

Experimental animals. Male Sprague-Dawley albino rats (274 ± 3 g; Taconic Laboratories, Germantown, NY) were housed in standard vivarium cages (3–4 rats/cage) in a room maintained at 25 ± 1°C on a 12:12-light-dark cycle, and were given food (Purina rat chow) and water ad libitum. Rats were weighed within 24 h of their arrival and allowed to acclimatize for at least 1 wk before experimentation.

METHODS

Animal care and use were in accordance with the Guidelines of the National Research Council and were approved by the Institutional Animal Care and Use Committee.

Surgical and vibration procedures. Rats were assigned randomly into groups in which the tendons from their predominantly fast-twitch (F) or slow-twitch (S) muscles were manipulated. Rats in the experimental groups underwent muscle denervation (FD, n = 11), tendon vibration (FV, n = 11 and SV, n = 6), or both (FDV, n = 12). Control rats (FC, n = 5 and SC, n = 5) were sham operated but neither denervated nor vibrated. All rats were anesthetized by intraperitoneal injection of a cocktail of ketamine (70–100 mg/kg) and xylazine (8–10 mg/kg). Boosters of ketamine (0.1 ml sc) were administered as necessary to maintain an appropriate level of anesthesia. Surgical manipulation and/or vibration were carried out unilaterally in each group.

Denervated rats underwent full lower limb denervation in which the femoral, obturator, sciatic, peroneal, and sural nerves were isolated and cut. Nondenervated rats underwent the same surgical procedures in terms of skin incisions and nerve isolations, but no nerves were cut. The Achilles tendon was exposed and severed at its distal end, leaving a small piece of the calcaneus bone attached. In the fast-twitch muscle groups, the soleus tendon was identified and cut near its insertion on the calcaneus. The gastrocnemius and plantaris tendons were tied with surgical suture, which, in turn, was attached to a lever system used to generate the vibration protocols. In the slow-twitch muscle groups, all tendons except that of the soleus were cut near their insertions and the soleus tendon was attached to the lever system as described above.

Rats were placed on a circulating water heating pad at 37°C, and the operated limb of each rat was stabilized with a knee pin (placed into the distal end of the femur) and foot clamp to prevent movement during the vibration procedure. The skin was used to contain a pool of warm mineral oil at the surgical site to prevent drying of the exposed tissue. This oil pool was maintained at ~36°C with a heat lamp equipped with a feedback mechanism. The suture tied to the isolated tendon was anchored by the calcaneus chip at the distal end and was attached to a force transducerlever system (model 305, Cambridge Instruments, Taunton, MA) such that the muscles were held approximately at their physiological resting length (~50 g of passive tension for the gastrocnemius and plantaris and ~10 g of passive tension for the soleus).

In the vibrated groups, a 150-Hz sinusoidal vibration stimulus was applied continuously to the tendon, perpendicular to the longitudinal axis of the muscle, for 15 min. This paradigm preferentially activates type Ia afferent fibers from muscle spindles (10, 11, 26). The stimulus was generated using a function generator and was monitored by using a Tektronix 2224 oscilloscope. A calibration curve was generated at the beginning of the experiment to establish the voltage-displacement relationship. The average amplitude of the oscillation was 1 mm from resting length (2 mm peak-to-peak displacement), and the average peak force generated for the highest level of stretch was 200 g for the gastrocnemius and plantaris muscles and 50 g for the soleus. Calibration was performed for the gastrocnemius and plantaris at the end of each experiment, showed ~50 g resting tension with ~200 g peak tension during the first oscillation, ~180 g of tension for the remainder of the oscillations in the train, and a displacement of ~0.85 mm per oscillation. The tendons of rats in nonvibrated groups were held at resting tension for 15 min.

Sample collection. Immediately after the 15-min experimental manipulation, rats were bled by cardiac puncture, with heparin as an anticoagulant, and decapitated. Blood samples were centrifuged (1,000–1,500 g, 30 min, 4°C) and ~1-ml aliquots of plasma from each rat were drawn off, stored at ~70°C with sodium fluoride as a preservative until used for determination of plasma hormone concentrations (GH, thyroxine (T4), and corticosterone (Cort)) by radioimmunoassay. The remaining plasma from each rat was pooled by experimental group and stored at ~70°C without preservative until used for determination of BGH concentrations by bioassay. Anterior pituitary glands were removed from the sela turcica, pooled by group, and frozen at ~70°C. For hormone analyses, the glands were thawed, weighed, homogenized in a small volume of 0.01 M Na2CO3 in an all-glass hand homogenizer, and diluted to a 2 mg/ml stock solution, on the basis of pituitary wet weight, with 0.85% NaCl for immunooassay.

Hormone assays. Measurements of BGH and GH were performed as described below, and T4 and Cort were immunoassayed from individual samples, in duplicate, using commercially available kits. T4 was measured by solid-phase (Diagnostic Products, Los Angeles, CA) and Cort by double-antibody (ICN Biomedicals, Costa Mesa, CA) immunoassay. Intra- and interassay coefficients of variation were <10% for the kit immunoassays, and the limits of detection are 0.25 μg/dl for T4 and 7.7 ng/ml for Cort.

A variation of the double-antibody immunoassay procedure of Schalch and Reichlin (35) was used for measurement of plasma and pituitary GH. Reagents were generously provided by Dr. A. Parlow at the Harbor-UCLA Medical Center (Torrance, CA) and the National Hormone and Peptide Program from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Purified rat GH (NIDDK-rGH-I-7; 2.0 IU/mg) was iodinated and served as a competitive inhibitor of endogenous GH. A reference preparation (NIDDK-rGH-RP-2) was used to generate a standard curve. The primary antibody (NIDDK-anti-rGH-S-5) was used at a final dilution of 1:3,000, and the final dilution of the secondary antibody (goat anti-mouse gamma globulin, Antibodies, Davis, CA) was 1:15. Plasma GH was assayed directly, in duplicate, from individual samples. For pituitary GH measurements, the 2 mg/ml stock homogenates were diluted 1:3,000 with 0.85% NaCl to fall within the sensitivity of the assay (0.8 ng/ml limit of detection) and assayed in quadruplicate. For this assay, the intra- and interassay coefficients of variation were ~4 and ~6%, respectively.

Plasma and pituitary BGH concentrations were assayed according to the method of Greenspan et al. (20). Briefly, young female rats, hypophysectomized during a phase of rapid linear growth (26 days of age), were injected with 0.85% NaCl, a purified bovine pituitary GH standard (XIV-44-C5, 1.5 IU/mg; 5, 15, or 45 μg total dose), or pooled plasma or pituitary samples from experimental rats. Assay rats were given a total dose of 2 mg/kg to each of plasma, while pituitary samples were assayed at two dose levels by injecting homogenate solutions diluted to deliver total doses of 7/5 or 7/5 mg tissue per assay rat in a 2-ml volume. Standards and samples were injected intraperi-
tonally (0.5 ml sample- rat-1-day-1) for 4 days, and rats were killed
by CO2 overdose on day 5. One tibia was dissected, split longitudi-
nally, and stained with AgNO3 to distinguish the noncalcified epiph-
yseal growth plate (the “silver line”) from surrounding calcified tissue.
Ten width measurements were taken across each epiphysis and aver-
aged, giving mean tibial plate width for each assay rat, which were
compared against the standard curve (Fig. 1) to obtain individual BGH
values. Group BGH values (means ± SE) then were calculated, and it
is these values that are reported (n = 3-6/group). Because the specific
biological activity (IU/mg) of highly purified rat GH is twice that of
the bovine standard (39), values obtained from the standard curve
were divided by two. Plasma BGH is, therefore, expressed as micro-
grams per milliliter of rat pituitary GH equivalents, and pituitary BGH
is reported as micrograms rat pituitary GH equivalents per milligram
of pituitary tissue. This method of measurement is extremely consis-
tent, showing tibial width increases of a similar nature across groups
of assay rats injected with the same sample, which then can be
converted to BGH levels in rat GH equivalents.

Statistical methods. Individual plasma GH, T4, and Cort measure-
ments were analyzed by using one-way ANOVA to determine overall
differences, followed by Scheffé’s post hoc test to determine group
differences (StatView, Abacus Concepts, Berkeley, CA). The vari-
ances in pituitary GH assay results were measured by ANOVA.
Plasma BGH measurements (1 dose level) were analyzed by using a
bracketed three-point assay method, and pituitary BGH measurements
(2 dose levels) were analyzed with a four-point assay procedure (37),
and between-group differences in BGH concentrations were deter-
mined by ANOVA. The measurements given for BGH reflect the
responses of the bioassay rats (n = 3-8/group) to pooled samples
from the experimental rats, rather than the responses of individual
experimental rats (n = 5-12/group) to the treatment they received.
For all measurements, power calculations yielded values of ≈80%,
and significance was determined at the P < 0.05 level.

RESULTS

The standard curve for the tibial bioassay, used to determine
sample BGH concentrations, is shown in Fig. 1. Epiphyseal
growth plates are measured by immunoassay. (Saline = 156.2)

![Fig. 1. Bioassayable growth hormone (BGH) bioassay standard curve. Purified bovine pituitary growth hormone (GH) induces a dose-dependent widening of the epiphyseal plate in the tibias of hypophysectomized rats. Total doses of 0 (saline), 5, 15, and 45 μg of GH were administered to hypophysectomized female rats (n = 5/group). Tibial epiphyseal widths (“silver line”) of all animals per group are plotted against the log of the dose, and the best-fit line and correlation calculated. The line equation then is used to determine the effective BGH dose in plasma and pituitary samples administered to separate groups of rats. At lower GH doses (<1.67 μg) the curve becomes nonlinear, as indicated by the dashed portion of the fit line. In the slow-twitch muscle vibrated group, 3 of 5 rats had epiphyseal widths falling below this demarcation on the curve, resulting in BGH values that could only be approximated by the line equation but that were significantly greater than in the saline-injected controls.

![Table 1. Other hormone concentrations measured by immunoassay]

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma GH (ng/ml)</th>
<th>Pituitary GH (μg/mg tissue)</th>
<th>Plasma T4 (μg/dl)</th>
<th>Plasma Cort (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FD</td>
<td>22.3±7.5</td>
<td>31.8±2.3</td>
<td>2.0±0.2</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>FV</td>
<td>23.4±7.3</td>
<td>30.5±1.0</td>
<td>1.8±0.2</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>FDV</td>
<td>6.8±2.7*</td>
<td>21.9±1.3*</td>
<td>1.6±0.1</td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE of concentrations of other hormones in the plasma and pituitary of rats after manipulation of their gastrocnemius and plantaris tendons. F, fast; D, denervated; V, vibrated; GH, growth hormone; T4, thyroxine; Cort, corticosterone. *Significantly different from FD and FDV, P < 0.05.

![Fig. 2. Plasma and pituitary BGH responses to gastrocnemius and plantaris tendon vibration. BGH concentrations were measured in the plasma of non-
denervated, nonvibrated control rats (FC, n = 3) and in rats that were
denervated (FD, n = 7), had their gastrocnemius and plantaris tendons vibrated for 15 min (FV, n = 6), or were denervated and vibrated (FDV, n = 8). BGH
also was measured in the pituitary tissue of these animals (n = 5/group).
Values are means ± SE. *Significantly different from FC, P < 0.05.

widths in hypophysectomized rats (n = 5/group) injected with
saline (vehicle) averaged 156.2 ± 1.0 μm, while those injected
with the bovine GH standard increased dose dependently:
186.2 ± 1.2, 212.0 ± 0.4, and 235.8 ± 3.1 μm for 5-, 15-, and
45-μg doses, respectively. The dose-response curve (r = 0.982),
yielded by graphing the data on a semilog plot across
experiments, is linear between dose levels of 1.67 and 45 μg of
bovine GH, and all of our sample data fell within this portion of
the graph.

Vibration of the tendons from fast ankle extensors stimu-
lated BGH secretion in a rapid and specific fashion (Fig. 2).
Fifteen minutes of vibration in nondenervated (FD) rats in-
creased plasma BGH to 260% of control (FC) values (6.11
± 0.44 vs. 2.35 ± 0.06 μg/ml; P = 0.001), with a concomitant
61% decrease in pituitary BGH concentration (11.90 ± 0.37
μg/mg; P < 0.001). No changes were seen in plasma or pituitary GH in response to denervation alone (FD). Full lower
limb denervation ablated the BGH response to fast muscle tendon vibration (FDV), and plasma BGH was
16% lower (1.98 ± 0.08 vs. 2.35 ± 0.06 μg/ml; P = 0.02) in
these compared with FC control rats.

The effects of fast muscle tendon vibration on BGH release
from the pituitary were selective, because the levels of other
plasma hormones were decreased or unaffected by vibration (Table 1). GH concentrations measured by radioimmunoassay
were higher than expected in the plasma of FD (22.3 ± 7.5
μg/ml), FV (23.4 ± 7.3 μg/ml), and FDV (6.8 ± 2.7 μg/ml).
ng/ml) and FDV (23.4 ± 7.3 ng/ml) rats, but they were not different between these groups. In response to fast muscle tendon vibration (FV), plasma GH concentrations were 6.8 ± 2.7 ng/ml, significantly lower than FD or FDV rats (P < 0.05). Pituitary GH concentrations were not altered by any of these manipulations, nor were plasma T₄ or Cort levels. Unfortunately, a limited volume of sample was obtained from FC rats, and it was used in its entirety for BGH analyses, which precluded our ability to assess GH, T₄, or Cort levels by immunoassay in the FC rats. However, useful interpretations still can be made (see DISCUSSION).

In marked contrast to the results from fast muscle tendon vibration, vibration of the soleus tendon alone inhibited BGH secretion (Fig. 3). Compared with control (SC) rats, vibrating the tendon of this slow ankle extensor for 15 min in nonde-nervated rats (SV) resulted in a 68% lower plasma (0.67 ± 0.03 vs. 2.07 ± 0.08 μg/ml; P < 0.001) and a 33% higher pituitary (42.57 ± 1.25 vs. 32.12 ± 1.35 μg/mg; P = 0.001), BGH concentration.

DISCUSSION

BGH release in response to tendon vibration. The present data provide evidence that activation of group Ia afferents from the primary endings of muscle spindles in fast muscles triggers BGH secretion from the pituitary in the rat. Activation of spindle afferents from a slow muscle, in contrast, suppresses BGH release. This pattern is consistent with previous data from our laboratory, showing increased BGH secretion after short bouts of exercise (4) or electrical stimulation of rat hind-limb muscle nerves (19) and decreased secretion after slow muscle nerve stimulation (18). In humans, McCall et al. (30) showed an enhancement of BGH release after vibration of the tibialis anterior muscle and no effect after soleus muscle vibration. Because the tibialis anterior in humans is comprised predominantly (~75%) of slow fibers (16), these results suggest that there may be aspects of neuromuscular activation related to muscle function (flexor, extensor) as well as type (fast, slow) that are important in the modulation of BGH release. In rats, the magnitude of the BGH response to fast muscle tendon vibration was similar to that after treadmill exercise (4) or tibial nerve stimulation (19). Combined, these results suggest that the secretion of BGH in response to neuromuscular activity during locomotion is mediated primarily by group Ia fiber activation from muscles that are composed predominantly of fast fibers. Furthermore, all evidence is consistent with the interpretation that BGH release is suppressed when only the spindles from a slow extensor muscle are activated. Alternatively, the ability of group Ia fiber inputs to modify BGH secretion may be a function of the mass of muscle and, therefore, the total number of spindles, activated. This is likely not the case, however, because previous studies by our laboratory have shown similar increases in BGH secre-tion in rats after unilateral stimulation of the sciatic, peroneal and/or tibial nerves, each of which would comprise afferents from large and varying amounts of skeletal muscle mass (19). Furthermore, excitation of afferents from multiple muscle groups of different types through treadmill exercise in rats induced BGH release of a similar magnitude as the nerve stimulation studies (4). Lastly, the soleus muscle has the highest spindle density of any hindlimb muscle (E. Eldred, personal communication), suggesting that stimulation of the entire triceps surae should induce more BGH release than stimulation of only fast muscle nerves if the activation of BGH secretion were dependent on the number of spindles activated. This was not seen in our studies.

Whereas BGH secretion was altered in response to acute changes in neuromuscular activity, neither plasma nor pituitary BGH was affected by acute denervation alone. This indicates that the BGH release is not dependent on activity-independent neuromodulatory influences from the intact nerve. Fast tendon vibration in denervated rats did not affect pituitary BGH. Decreases in plasma BGH in these rats suggests that, in the absence of release, BGH is inactivated or cleared from the circulation over time. Furthermore, these data demonstrate that the vibration stimulus influences BGH release exclusively through neural pathways and that it is not transmitted indirectly via other tissues, e.g., bone and muscle.

In addition to acute neuromuscular activation, chronic activity levels also are implicated as a factor in this neurally mediated control of BGH secretion. Chronic unloading through bed rest or spaceflight in humans suppresses the BGH response to exercise, whereas the basal BGH concentrations remain normal (28, 29). Moreover, the exercise-induced release of BGH is restored after a few days of normal reambulation (28, 29).

Consistent with previously published data from our laboratories (4, 18, 19), the effects of neuromuscular activation on hormone secretion appear to be specific to BGH. Pituitary GH concentrations were unchanged in response to fast muscle tendon vibration in nondenervated rats (FV), and the low plasma GH levels in this group argue against a specific release effect on this hormone. In contrast, the higher plasma GH concentrations in both of the denervated groups (FD and FDV) suggest that circulating GH levels may be altered by denervation and/or increased surgical manipulation. The absence of GH data from FC rats, however, precludes an accurate evaluation of the effects of denervation on GH secretion, which will need to be addressed in future experiments. Plasma T₄ and Cort concentrations were similar among all groups tested. However, elevations in plasma testosterone and GH levels have been reported after vibration in men (6). This apparent discrepancy may be related to differences in the subjects or the number of
afferent fibers activated with each experimental paradigm. For example, Bosco et al. (6) studied the hormonal responses to whole body vibration using an externally applied vibration stimulus in men, whereas our vibration paradigm involved the vibration of the tendon of a single muscle or muscle complex within one ankle extensor compartment in the rat.

Proposed pathway for afferent modulation of GH secretion. The results of the present and previous studies (18, 19, 30) suggest that BGH release from the rat pituitary is modulated by Ia afferent inputs from working skeletal muscle. The spinal and supraspinal projections through which this modulation occurs remain to be determined, but a possible pathway is through the hypothalamic locomotor region. This region includes the lateral and posterior hypothalamic areas, which are ideally situated to exert an influence on hypothalamic and pituitary hormone release (3, 24, 40). Electrical stimulation of the lateral hypothalamic nucleus elicits locomotor activity in rats (36). Excitatory responses have been recorded in the cat hypothalamic locomotor region, especially in the posterior hypothalamus, after static or dynamic triceps surae muscle contraction induced by electrical stimulation or with mechanical probing of the musculature (40). Neuronal activation, in the form of immediate-early gene expression, has been demonstrated in the hypothalamic locomotor region after treadmill exercise in rats (24). Toney and Mifflin (38) have shown that mechanoreceptor activation in the hindlimbs of rats elicits neuronal responses in the nucleus of the solitary tract, a region known to project to the hypothalamic locomotor region (3). Lastly, a large number of direct projections from the spinal cord to regions of neuroendocrine control in the medial and lateral hypothalamic have been demonstrated (8). Together, these results illustrate potential pathways for peripheral muscle afferent inputs to reach the anterior pituitary gland and modulate endocrine output.

Alternatively, other pathways may play a role in mediating the control of BGH secretion. The divergence of Ia fibers is extensive. Each muscle spindle projects afferents to almost every homonymous motoneuron, and as many as 80% of the motoneurons of synergistic muscles can receive input from that single muscle spindle (9). Furthermore, there is a similar extensive divergence to spinal interneuron projections, such as Ia inhibitory neurons projecting to antagonistic motor pools (9). The activation of a muscle, therefore, results in an extensive level of broadcasting of that event, potentially to many pathways projecting to the brain.

Contrasting effects of slow vs. fast extensor spindles. Given the differential effects of afferent inputs from fast and slow muscle on BGH release in rats (this study and Ref. 18), these inputs must diverge at some point. However, convergence of the signals must occur at some level before reaching the pituitary, because BGH secretion is increased similarly when only fast muscles are activated and when fast and slow muscles are activated simultaneously (4, 19). It is our interpretation that this differential activation is relevant to the demands placed on the body. Recruitment of large muscle groups occurs during situations of increased work production that stress the metabolic and structural integrity of the body. BGH may play a role in the growth and or maintenance of the musculoskeletal system under these conditions. Alternatively, BGH secretion appears to be rapidly and markedly increased in response to any stimulus that challenges the body’s energy supply, such as exercise (4), fasting, or insulin-induced hypoglycemia (14). Its major function may, therefore, be to protect the central nervous system by mobilizing other resources for use by active tissues. Slow muscle activation, in contrast, is near maximal when simply maintaining an upright posture. It seems unlikely that the release of an anabolic growth factor would benefit the body under such low levels of activation. Clearly, further experiments will be required to identify the neural pathways for the muscle afferents that modulate BGH release.

Functional significance. Whereas BGH has yet to be fully isolated and characterized, results from multiple experiments have demonstrated that it is a hormone or growth factor of anterior pituitary origin and that it promotes hyper trophy and hyperplasia in the chondrocytes of long bones of hypophysectomized young rats (4, 13, 18–23, 28–30, 33). In this capacity, basal plasma BGH in rats has a growth-promoting activity equivalent to ~2 μg/ml of GH. However, basal circulating levels of GH most often are on the order of only ~10 ng/ml (1, 2, 13). Other possible functions of BGH remain to be determined but are likely to be related to the overall maintenance and/or strengthening of the musculoskeletal system. In support of this hypothesis, Rubin et al. (34) showed that high-frequency, low-magnitude vibration increased femur density in sheep, suggesting that vibration may be a noninvasive method for the treatment of osteoporosis. Falempin and In-Albon (15) have shown that twice daily vibration (48 s; 120 Hz) of the stretched Achilles tendon ameliorated the decreases in soleus muscle mass and type IIA and IIC fiber cross-sectional areas in hindlimb-unloaded rats, indicating that vibration may be an appropriate countermeasure to the atrophy induced by chronic non-weight bearing. In another study, 2 days of an external vibration stimulus (5 h/day) increased plantar muscle mass and type I and IIC fiber cross-sectional areas in normal, anesthetized female rats (31). Bosco et al. (5) reported that whole body vibration in women enhanced their velocity, force, and power production in a resistance exercise test, a result the authors attributed to unidentified “neural factors.” Whether BGH plays a role in these anabolic effects remains to be determined.

In conclusion, we have provided evidence that the muscle afferent-pituitary axis can act rapidly to modulate BGH secretion in response to the activation of skeletal muscle afferents that are likely to be the Ia afferents of muscle spindles. This axis may provide a real-time link between the amount of activity performed by muscles associated with posture and locomotion and the release of a factor related to musculoskeletal integrity.

ACKNOWLEDGMENTS

The authors thank Dr. John Hodgson for assistance in the vibration paradigm; Dr. A. F. Parlow and the National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Peptide Program for the GH immunoassay reagents; and Lisa Baer, Megan Moran, and Tom Wang for assistance in performing the bioassays.

Present address of K. L. Gosselink: Laboratory of Neuronal Structure and Function, The Salk Institute, 10010 N. Torrey Pines Rd., La Jolla, CA 92037.

GRANTS

This work was supported by the National Aeronautics and Space Administration (NASA) Graduate Student Researchers Program (GSRP-98-104) and National Institute of Dental Research National Research Service Award DE-07212 (both to K. L. Gosselink). Other support included NASA Grant 199-26-12-09 (to R. E. Grindeland, V. R. Edgerton, and R. R. Roy).
REFERENCES


