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Muscle afferent activity modulates bioassayable growth hormone in human plasma

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McCall, G. E ., R . E . Grindeland, R. R. Roy, and V. R. Edgerton. Muscle afferent activity modulates bioassayable growth hormone in human plasma. *J Appl Physiol* 89: 1137–1141, 2000.—Immunoassayable and bioassayable growth hormone responses to vibration-induced activation of muscle spindle afferents of the soleus (Sol) or tibialis anterior (TA) muscles were studied in 10 men. Subjects were supine while a 10-min vibration stimulus (100 Hz; 1.5-mm amplitude) was applied to the muscle, with each of the muscles tested on separate days. Blood samples were collected before, during, immediately after, and after 5 and 10 min of vibration. Plasma growth hormone concentrations were determined by radioimmunoassay (IGH) for all sampling periods and by bioassay (BGH; measurement of tibial epiphysial cartilage growth in hypophysectomized rats) for samples obtained before and immediately after vibration. Plasma IGH concentrations were similar at all time points during the Sol or TA experiments. After 10 min of muscle vibration, mean plasma BGH was elevated 94% [1,216 ± 148 (SD) to 2,362 ± 487 μg/l; $P = 0.0001$] for TA and decreased 22% (1,358 ± 155 to 1,058 ± 311 μg/l; $P = 0.09$) for Sol. These data demonstrate that activation of TA muscle spindle afferents increases circulating BGH but not IGH. The absence of a similar vibration-induced BGH response for the Sol indicates a differential regulation of BGH release by these two predominantly slow muscles, perhaps related to their respective flexor and extensor functions. These data indicate that a muscle afferent-pituitary axis modulates the release of BGH, but not IGH, from the pituitary in humans and that this axis is muscle specific, similar to that observed in rats.

muscle spindle; vibration; soleus; tibialis anterior; extensor-flexor; slow-twitch muscle; fast-twitch muscle; proprioception; pituitary hormones

MULTIPLE VARIANTS OF growth hormone exist in the pituitary and circulation. Heterogeneity of pituitary growth hormone occurs by a variety of mechanisms, including posttranscriptional and posttranslational events, resulting in over 100 forms of circulating growth hormone (1). Although circulating growth hormone is commonly measured by immunoassay, a number of studies (6, 7, 11, 12, 15, 17–19, 22, 23) report a biologically active form(s) of growth hormone not measured by standard immunoassay. Our laboratory recently reported that a brief protocol of unilateral isometric plantar flexor contractions in humans increases plasma growth hormone concentration when measured by bioassay (BGH) but not by immunoassay (IGH) (22, 23). This response of BGH to exercise was inhibited by chronic muscle unloading during 17 days of bed rest (22) or spaceflight (23) and returned within 2–4 days of reloading. A variety of in vitro and in vivo data from rats have also shown that the release of BGH from the pituitary was inhibited by 14 days of hindlimb unloading (18) or by 7–14 days of spaceflight (15, 18, 19). Moreover, our laboratory recently demonstrated in rats that muscle afferent activity stimulated pituitary BGH release into the circulation and proposed that a muscle afferent-pituitary axis can modulate BGH release (11–13). Because spaceflight affects multiple aspects of neuromuscular function pertaining to proprioception in humans (4), the disruption of an afferent-pituitary axis modulating BGH release might be one explanation for the absence of an exercise-induced BGH response observed during spaceflight (23).

Given that low-threshold muscle afferents (12) and perhaps, more specifically, muscle spindles (13) have been reported to modulate BGH release in rats, the present study was designed to determine whether muscle spindle afferent activation modulates BGH plasma concentrations in humans. Muscle spindle afferents (primary and secondary) of the soleus (Sol; ankle extensor) or tibialis anterior (TA; ankle flexor) muscles were activated by high-frequency vibration, which has been considered to result in a greater sustained inflow from group Ia spindle afferents than any other type of physiological stimulus (3). Because isometric plantar flexor contractions elevate plasma BGH in humans (22, 23), we hypothesized that activation of Sol afferents would increase plasma BGH. However, because electrical stimulation of the proximal end of the Sol nerve inhibited BGH release in rats (11), an alternative hypothesis was that Sol afferent activity would not

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change, or perhaps even decrease, plasma BGH. Lastly, we hypothesized that activation of TA afferents would increase plasma BGH, an expectation consistent with the decreased pituitary and increased plasma BGH concentrations after electrical stimulation of the proximal end of the peroneal nerve in rats (12). No vibration-induced changes in IGH were hypothesized.

METHODS

Subjects were 10 healthy men, mean age of 26.7 ± 1.6 (SD) yr, without any known neuromuscular disorders. Subjects reported to the lab after an overnight fast, and all experiments occurred between 7 and 10 AM to minimize the normal rhythmic and diurnal fluctuations of IGH levels (24). During the experiments, the subjects lay on their side in a comfortable position on a padded surface with their right leg next to the pad. The ankle and knee joints of the vibrated (left) leg were positioned at ~110° with the use of a goniometer. Once positioned, the subject was asked to lie still and relax throughout the testing period. During each experimental session, the same procedures were followed, except that the TA and the Sol muscles were vibrated on different days in random order. All procedures were approved by the University Human Subjects Committee, and each subject signed a written, informed consent before participation.

Muscle vibration. The vibration site was located and marked on the skin overlaying the muscle. For the TA, the vibration site was one-third of the distance between the inferior border of the patella and the lateral malleolus and ~2 cm lateral to the tibia. The Sol vibration site was midline below the belly of the gastrocnemius muscle. These sites are the same as those used in our laboratory to record surface electromyography from these muscles and were chosen to isolate the vibration stimulus to the intended muscle as much as possible. A large custom-built motor controlled by a function generator was used to apply the vibration stimulus. The large size of the motor eliminated any dampening of the amplitude and frequency of the vibration stimulus. This was verified by monitoring the output of the motor on an oscilloscope when forces substantially greater than those encountered during the experiments were applied to the arm of the motor. The vibration stimulus consisted of a continuous 10-min train of vibration (1.5-mm amplitude at 100 Hz) applied to the surface of the skin overlaying the muscle. These vibration parameters have been used by Burke et al. (3) to activate primary (Ia) and secondary (II) muscle spindle afferents when using either tendon or muscle vibration in humans. In their studies, group Ia endings generally discharged 1:1 with the vibration frequency and exhibited a greater responsiveness to vibration than secondary endings, which discharged at subharmonic frequencies (3). The tested leg was held securely and with constant pressure, with a 2.5-cm circular aluminum disk attached to the motor arm and positioned on the marked site. For consistency, the same investigator (McCall) manually positioned and held the leg against the motor arm during all experiments. Manual application of the vibration stimulus to the muscle belly was used because this method allowed control of both the site of vibration and the pressure applied during vibration that was better than either strapping the limb to the vibrator (3, 10) or vibrating the muscle tendon (3). In addition, the latter two methods result in the spread of vibration beyond the desired muscle (3, 10).

Blood collection and growth hormone assays. After the subjects arrived at the laboratory, an in-dwelling flexible catheter was placed in an antecubital vein and kept patent with a heparin saline solution. After the catheter was inserted, the subjects rested 20 min before the resting (previbration) blood sample (20 ml) was obtained, to minimize any potential hormone fluctuations related to anticipatory responses of the catheter insertion (21). Additional blood samples (20 ml each) were obtained after 5 min of vibration, immediately at completion of 10 min of vibration (postvibration), and 5 and 10 min after vibration. The subjects remained supine during all blood-draw procedures. All blood samples were collected using heparin as an anticoagulant, placed on ice, and then centrifuged at 1,000 *g* for 30 min at 4°C. The plasma was removed, aliquoted, and stored at −70°C until the hormonal analyses were performed.

Both IGH and BGH were measured. Human IGH was measured using ^{125}I radioimmunoassay kits (Diagnostic Products, Los Angeles, CA). IGH assays were performed for each time point sampled. The complete sets of samples from the Sol and TA experiments were run in two separate assays. IGH intra-assay variances were 7.0 and 6.9% for the Sol and TA, respectively, and interassay variance was 4.8%. BGH was determined only for the samples from before and after vibration by using the 4-day bioassay of tibial epiphysial cartilage growth of Greenspan et al. (14) and as described previously (22). BGH assay variance was 2.0% and 1.7% for intra-assay and interassay, respectively.

Statistical analysis. Two (within-subject)-factor repeated-measures multivariate ANOVA (multiple linear regression model) was used to assess the effects of vibrating the Sol and TA muscles on the changes in IGH and BGH plasma concentrations. Because ANOVA post hoc tests are designed to compare the means of independent (i.e., uncorrelated) observations, they are not appropriate for a within-subject repeated-measures design, requiring pair-wise contrasts between groups to be specified a priori. Pair-wise contrasts were used to compare differences between pre- and postvibration IGH and BGH values for each muscle. All analyses were performed using SuperAnova statistical software (Abacus Concepts, Berkeley, CA) and Apple Macintosh microcomputers. Significant differences were established at $P \leq 0.05$.

RESULTS

BGH. Significant ANOVA main and interaction (muscle × sample time; $P = 0.0001$) effects for BGH were observed between TA and Sol muscles ($P = 0.0002$) and previbration and postvibration sample times ($P = 0.0001$). After 10 min of vibration of the TA, the mean plasma BGH increased 94% [1,216 ± 148 (SD) to 2,362 ± 487 μg/l; $P = 0.0001$; pair-wise contrast; Fig. 1]. The 22% decrease in mean plasma BGH after 10 min of vibration of the Sol approached significance [1,358 ± 155 (SD) to 1,058 ± 311 μg/l; $P = 0.09$; pair-wise contrast; Fig. 2].

IGH. Plasma IGH concentrations were similar across all sample times for Sol and TA experiments. To facilitate comparisons with BGH, only IGH values from samples before and after vibration are depicted in Figs. 1 and 2. No significant ANOVA main or interaction (muscle × sample time; $P = 0.71$) effects for IGH were observed between TA and Sol muscles ($P = 0.43$) or for previbration and postvibration sample times ($P = 0.36$). For TA vibration, the mean IGH concentrations before and after vibration were 1.53 ± 1.77 (SD) and 1.44 ± 1.37 μg/l, respectively (Fig. 1). For Sol vibration, the mean IGH concentrations before and

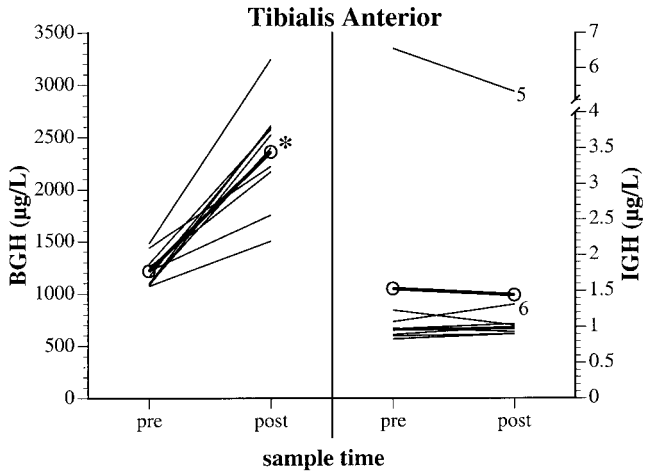

Fig. 1. Plasma bioassayable growth hormone (BGH; *left* *y*-axis) and immunoassayable growth hormone (IGH; *right* *y*-axis) responses before (pre) and after (post) a 10-min vibration of the tibialis anterior muscle. Data for individual subjects are depicted as thin lines and for group mean as a thick line with open circles at each end. *Subjects 5* and *6* are identified in the IGH graph. *Significant increase from pre for BGH, $P = 0.0001$.

after vibration were 3.54 ± 7.87 (SD) and 3.53 ± 7.57 μg/l, respectively (Fig. 2). The IGH absolute values for the majority of subjects were remarkably similar (Figs. 1 and 2). The large standard deviations were due to *subjects 5* and *6*, who exhibited consistently elevated IGH concentrations for the TA and Sol experiments, respectively. However, on the day that these subjects performed the experiment for the other muscle, their IGH values were comparable to the majority of subjects. When these outlier values were excluded, the mean results of the remaining nine subjects were similar [Sol: 0.97 ± 0.12 (SD) previbration and 1.01 ± 0.12 μg/l postvibration; TA: 1.06 ± 0.30 (SD) previbration and 1.14 ± 0.38 μg/l postvibration].

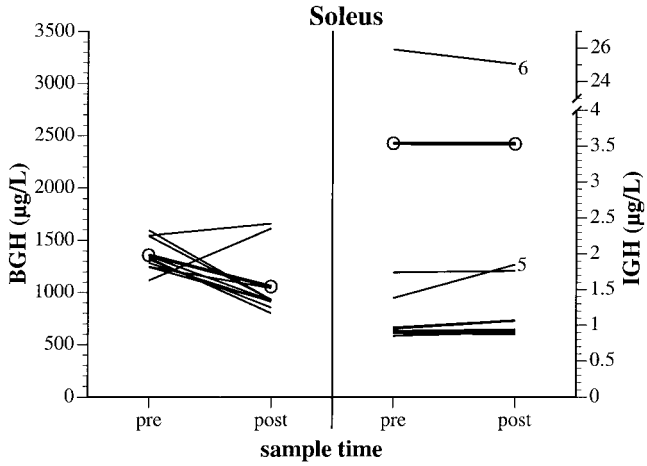

Fig. 2. Plasma BGH (*left* *y*-axis) and IGH (*right* *y*-axis) growth hormone responses before (pre) and after (post) a 10-min vibration of the soleus muscle. Data for individual subjects are depicted as thin lines and for group mean as a thick line with open circles at each end. *Subjects 5* and *6* are identified in the IGH graph.

DISCUSSION

The results of the present study provide the first evidence in humans that activation of muscle spindle afferents via vibration modulates plasma BGH concentrations. These data in humans are consistent with those previously reported by Gosselink et al. (11, 12) with low-threshold muscle nerve stimulation in rats in which in situ stimulation of the proximal stump of a severed nerve (peroneal) innervating dorsiflexor (TA, extensor digitorum longus, peronei) muscles increased plasma BGH and decreased pituitary BGH proportionally (12). In contrast, stimulation of the Sol nerve decreased plasma BGH (11). Gosselink et al. (11, 12) hypothesized that the differential BGH responses between stimulation of the peroneal and Sol nerves was related to their respective innervation of predominantly fast-twitch (TA, extensor digitorum longus, peronei) and slow-twitch (Sol) muscles. In humans, however, the TA and Sol are comprised of ~75% and ~80% slow fibers, respectively (9, 20). Therefore, the muscle-specific BGH response to afferent activation in humans may be related to the flexor or extensor function rather than the fiber type composition of the muscle. However, Gosselink et al. (12) suggested that the differences in BGH response in rats were not related to the flexor or extensor function of the muscle because proximal stimulation of the severed tibial nerve that innervates the plantar flexor compartment, which consists of predominantly slow (Sol) and predominantly fast (gastrocnemius, plantaris) muscles, also increased plasma BGH. Thus, although afferent stimulation modulates plasma BGH in both humans and rats, differences in muscle fiber type composition between species preclude the formulation of a clear consensus regarding the importance of myosin phenotype specificity in defining the BGH responses.

The tendency for Sol muscle spindle activation to decrease plasma BGH in the present study is consistent with the results from the rat nerve-stimulation experiments described above (11). However, the lack of a Sol BGH response to vibration in humans is perplexing, given that plasma BGH has been shown to increase after a 4- to 6-min protocol of isometric plantar flexions (22, 23). The exercise protocols consisted of a series of submaximal contractions interspersed with an occasional pair of maximal efforts, with all contractions performed sequentially and at a 4-s to 1-s contraction-relaxation interval. During an isometric contraction, ~75% of the muscle spindle afferents are activated, and there is also a burst of spindle discharge on abrupt relaxation (5). Therefore, the exercise protocols used in these prior studies (22, 23) would have elicited a considerable activation of Sol (and other plantar flexor) muscle spindle afferents. The reason(s) that the vibration-induced activation of the Sol muscle spindles in the present study did not increase plasma BGH is unclear. Perhaps isometric co-contractions of the predominantly fast agonists (medial and/or lateral gastrocnemius) during plantar flexion can provide sufficient afferent activation to stimulate the exercise-

induced BGH release and thus override the tendency for inhibition from Sol afferents. This interpretation is consistent with the elevated plasma BGH that occurs in rats after either afferent nerve stimulation of the tibial nerve innervating the entire plantar flexor compartment (11, 12) or after running exercise in which all plantar flexor, as well as other hindlimb muscles, are contracting (2). Because the gastrocnemius tendon overlays the Sol muscle, gastrocnemius muscle spindle afferents were likely to have been activated at subharmonic frequencies during vibration of the Sol muscle in the present study. Whatever level of gastrocnemius afferent activity occurred during Sol muscle vibration, it was not sufficient to cause an increase of plasma BGH by overriding a potentially negative or absent effect of Sol afferent activity. Given the significant plasma BGH increases after TA vibration, it also is conceivable that a low-level antagonist co-contraction of the TA during plantar flexion provided sufficient afferent activation to elevate BGH in previous human exercise studies (22, 23). Lastly, we cannot rule out the possibility that plasma BGH was declining during the present experiment, and its tendency for decline after Sol vibration merely reflects the absence of a stimulatory effect. Regardless, it appears that, if muscle spindles represent a major source of neural activation modulating BGH release, then whether the spindle activation stimulates or inhibits release reflects a muscle-specific divergence of either excitatory or inhibitory spindle projections to the pituitary. Further studies could address these issues by testing the vibration of the medial and/or lateral gastrocnemius alone, and together with the Sol, by testing the simultaneous vibration of the Sol and TA and by including a nonvibrated control group to rule out a decline in plasma BGH over the time course of the experiment.

Perspective on BGH regulation and microgravity. Several rat studies have reported that spaceflight or hindlimb unloading decreases BGH secretion by pituitary somatotrophs in vitro and in vivo (15–19). In addition, human studies have shown that the normal exercise-induced plasma BGH increases that occur at 1 G are inhibited by spaceflight (23) or bed rest (22). Moreover, recent evidence indicates that BGH can be modulated by muscle afferent activity in rats, and a muscle afferent-pituitary axis for BGH regulation in rats has been proposed (11, 12). The significance of the proposed muscle afferent-pituitary BGH regulatory axis to spaceflight studies is that exposure to microgravity alters many aspects of neuromuscular proprioception (4). Thus the effects of spaceflight on BGH secretion could be related to altered proprioceptive input via this proposed muscle afferent-pituitary axis. The absence of an exercise-induced BGH response in humans during spaceflight (23), combined with the present results showing a muscle (spindle) afferent-induced BGH response, indicates that altered proprioceptive input during spaceflight may have contributed to a decreased capacity to secrete BGH in response to exercise. In addition, because cultured rat somatotrophs flown in space secrete less BGH after return to 1 G (19), microgravity may also have direct cellular effects on the capacity of human somatotrophs to produce and/or secrete BGH in response to exercise. Whether the altered BGH release is related to the skeletal muscle atrophy, bone demineralization, or other physiological adaptations that occur during spaceflight remains to be determined. However, vibration of the Achilles tendon was recently reported to attenuate atrophy of the Sol during hindlimb unloading in rats (8); it is intriguing to speculate that this attenuation of muscle atrophy was mediated, in whole or part, by an increase in circulating BGH via vibration-induced activation of a muscle afferent-pituitary axis.

Direct evidence for the existence of a muscle afferent-pituitary axis, as well as the molecular characterization of the BGH molecule(s), remains to be determined, as does the mechanism(s) and physiological significance of the altered BGH regulation by microgravity. We do know that there is an inverse relationship between pituitary and plasma BGH levels in rats over a wide range of experimental paradigms that either stimulate or inhibit its release (2, 11–13). We also have found that stimulating muscle afferents in hypophysectomized rats does not increase plasma BGH (Grindeland, Roy, and Edgerton, unpublished observations). In conclusion, although many questions remain unanswered, an accumulating body of evidence indicates that BGH is a potent, and perhaps novel, pituitary growth factor with a unique regulatory mechanism. Current efforts aimed at developing a less costly and less time-consuming BGH assay compared with the tibial bioassay should greatly facilitate both the determination of the regulatory mechanism(s) controlling BGH release and the isolation and characterization of the BGH molecule(s).

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