Skeletal muscle afferent regulation of bioassayable growth hormone in the rat pituitary


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Gosselink, K. L., R. E. Grindeland, R. R. Roy, H. Zhong, A. J. Bigbee, E. J. Grossman, and V. R. Edgerton. Skeletal muscle afferent regulation of bioassayable growth hormone in the rat pituitary. J. Appl. Physiol. 84(4): 1425–1430, 1998.—There are forms of growth hormone (GH) in the plasma and pituitary of the rat and in the plasma of humans that are undetected by presently available immunoassays (iGH) but can be measured by bioassay (bGH). Although the regulation of iGH release is well documented, the mechanism(s) of bGH release is unclear. On the basis of changes in bGH and iGH secretion in rats that had been exposed to microgravity conditions, we hypothesized that neural afferents play a role in regulating the release of these hormones. To examine whether bGH secretion can be modulated by afferent input from skeletal muscle, the proximal or distal ends of severed hindlimb fast muscle nerves were stimulated (~2 times threshold) in anesthetized rats. Plasma bGH increased ~250%, and pituitary bGH decreased ~60% after proximal nerve trunk stimulation. The bGH response was independent of muscle mass or whether the muscles were flexors or extensors. Distal nerve stimulation had little or no effect on plasma or pituitary bGH. Plasma iGH concentrations were unchanged after proximal nerve stimulation. Although there may be multiple regulatory mechanisms of bGH, the present results demonstrate that the activation of low-threshold afferents from fast skeletal muscles can play a regulatory role in the release of bGH, but not iGH, from the pituitary in anesthetized rats.

immunoassay; bioassay; proprioception; electrical stimulation; peripheral nerves; low-threshold afferents

GROWTH HORMONE (GH) is the most abundant hormone in the anterior pituitary gland of nonlactating mammals (18) and consists of a family of >100 molecular forms that vary in size and possibly function (2–4, 28). Variants of GH range from fragments as small as 5 kDa to large-molecular-weight forms (“big” and “big big” GH) which can be >100 kDa in size (3, 4, 18, 28). The 22-kDa product of the GH-N gene is the GH most commonly measured by immunoassay, and it is the form made by recombinant DNA technologies and used therapeutically for a variety of growth-related disorders. However, the degree of interaction between 22-kDa GH antibodies and GH variants or GH molecules bound to their binding proteins is largely unknown, and the likelihood is low that these antibodies consistently recognize large-molecular-weight GH forms.

It appears that the smaller GH variants are fragments of 22-kDa GH, or of a 20-kDa GH molecule that is alternatively spliced from the GH-N gene through the removal of part of exon 3 (2, 3), and that they result from proteolytic cleavage. However, the origin of large-molecular-weight variants of GH is less well understood. Posttranslational modification of the GH molecule, through glycosylation, complexing of GH (20 or 22 kDa) with binding proteins, or dimerization or oligomerization of smaller GH molecules, has been identified as one possible source (2–4).

The regulation of 22-kDa GH is mediated by hypothalamic GH-releasing hormone and somatostatin, and its physiology has been well characterized. In contrast, the physiological effects and regulatory mechanisms of large-molecular-weight variants of GH are less well understood. In the present study, we use changes in tibial epiphysial width in hypophysectomized rats as an in vivo bioassay to measure GH activity (13). These bioassayable GH (bGH) activity levels measured in rat plasma and pituitary tissue, as well as in human and bovine plasma, are largely independent of the levels of GH as measured by immunoassay (iGH) (9–11, 13, 17–19).

Secretion of bGH by pituitary cells cultured from rats exposed to spaceflight or by rat pituitary cells flown in space in culture is consistently decreased (~50%), whereas the iGH response is variable (17–19). These changes in bGH occur in the presence of normal levels of plasma metabolites that are thought to regulate iGH secretion. We hypothesized that, because proprioceptive feedback and sensory neurons are markedly disrupted after spaceflight (8, 21, 24), proprioceptive input may play a role in the regulation of bGH release. The purpose of the present study, therefore, was to determine whether bGH release from the pituitary could be regulated by afferent input from the hindlimb musculature. We found that bGH, but not iGH, was released from the rat pituitary when low-threshold afferents in the proximal end of severed nerves from fast muscle were stimulated.

METHODS

Experimental animals. Male albino rats (~200 g body wt; Taconic Sprague Dawley, Germantown, NY) were housed in shoebox cages (2–3 rats/cage) in a room maintained at 25 ± 1°C on a reversed 12:12-h light-dark cycle. They were given food (standard Purina rat chow) and water ad libitum. Rats were weighed ~24 h after their arrival, and they were allowed to acclimatize after shipment for at least 1 wk before experimentation. Animal care and use were in accordance with the Guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee.

Stimulation protocol. Peripheral nerves in the hindlimbs (sciatic, tibial, peroneal, and/or sural nerves) were surgically isolated and severed in rats that were deeply anesthetized.
(pentobarbital sodium, 50 mg/kg ip; n = 3–10/group). The proximal or distal nerve trunk was placed on a bipolar silver electrode and was stimulated in situ with a 20-μs square-wave pulse for 15 min at a train frequency simulating electromyographic patterns recorded from rats running on a treadmill at 40 m/min (100 Hz, 150 ms on:150 ms off) (25). The strength of the current was two times the threshold required to elicit a visible reflex response. In a separate experiment, the threshold level was tested further by recording the compound action potentials when antidromically stimulating the tibial nerve and recording proximally from the sciatic nerve. This current strength was sufficient to excite group I and some group II axons (i.e., afferents from Golgi tendon organs and primary and secondary endings of muscle spindles). The level of stimulation was well below that which excites group III and IV afferents, i.e., more than five times threshold. Control rats were prepared identically, but they were not stimulated. Blood and pituitary glands were collected immediately after the 15-min period and were handled as described below.

Sample collection. All rats were bled by cardiac puncture, with heparin used as an anticoagulant. Animals were then decapitated. After the blood was centrifuged (1,500 with heparin used as an anticoagulant. Animals were then handled as described below. With sodium fluoride as a preservative, in cryovials at −70°C for immunoassay of hormones and measurement of metabolites. Measurement of iGH was performed as described below, whereas other plasma hormones were immunoassayed by using commercially available kits. Testosterone, thyroxine, and triiodothyronine were measured by solid-phase immunoassay (Diagnostic Products, Los Angeles, CA); corticosterone was measured by double-antibody immunoassay (ICN Biomedicals, Costa Mesa, CA). Inter- and intraassay coefficients of variation were <10% for all kit immunoassays. Plasma metabolites (glucose, lactate, and triglycerides) were measured by using a COBAS automated analyzer (Roche Diagnostics, Montclair, NJ). The remainder of the plasma was pooled by treatment group and stored at −70°C until used for measurement of bGH. Anterior pituitary glands were cleanly dissected, pooled by group, and frozen at −70°C until used. The glands were thawed, weighed, homogenized in a small volume of 0.01 M Na2CO3 by using an all-glass hand homogenizer, diluted for bioassay with 0.85% NaCl, and measured for bGH concentrations.

GH assays. iGH was measured by using a variation of the double antibody procedure of Schalch and Reichlin (26). The rat GH (VII-38-C; 3 U/mg) that was used for standards and radiiodination with 125I and the polyclonal antiserum were double antibody procedure of Schalch and Reichlin (26). The remainder of the plasma was pooled by treatment group and stored at −70°C until used for measurement of bGH. Anterior pituitary glands were cleanly dissected, pooled by group, and frozen at −70°C until used. The glands were thawed, weighed, homogenized in a small volume of 0.01 M Na2CO3 by using an all-glass hand homogenizer, diluted for bioassay with 0.85% NaCl, and measured for bGH concentrations.

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Fig. 1. Plasma and pituitary bioassay growth hormone (bGH; A) and plasma immunoassay growth hormone (iGH; B) concentrations in control rats and in rats after stimulation of proximal end of severed sural or tibial nerve (n = 8/group). Values are means ± SE. *Significantly different from control; P < 0.05.
stimulation of the proximal end of the tibial nerve (Fig. 1B). These data demonstrate that bGH, but not iGH, secretion can be modulated by afferent input from the hindlimb musculature. Similar changes were found after 5 or 10 min of stimulation (data not shown), emphasizing the rapidity of the bGH response to muscle afferent activation. In a separate experiment, the proximal end of the severed tibial nerve was stimulated in hypophysectomized and normal (pituitary intact) rats. This stimulation increased plasma bGH by ~100% in normal rats; bGH was undetectable in the plasma of both stimulated and unstimulated hypophysectomized rats (data not shown). Stimulation of the proximal end of the severed peroneal nerve, innervating primarily fast ankle flexors, gave a response similar to stimulation of the proximal end of the tibial nerve. Plasma bGH increased by 210% and pituitary bGH decreased by 66% (Fig. 2A), with no significant effect on plasma iGH (Fig. 2B). Furthermore, simultaneous stimulation of the proximal ends of the peroneal + tibial + sural nerves increased plasma and decreased pituitary bGH by ~250 and ~70%, respectively, a response similar to proximal stimulation of either the tibial nerve or peroneal nerve alone (Fig. 3A). Again, plasma iGH was unchanged from control levels (Fig. 3B). The response to stimulation of the proximal end of the sciatic nerve, innervating the entire lower hindlimb, approximated the results seen when the proximal tibial and/or peroneal nerves were stimulated (data not shown).

Sural and distal nerve stimulation. Stimulation of the proximal end of the severed sural nerve, a largely cutaneous nerve, had no effect on plasma or pituitary bGH (Fig. 1A) or plasma iGH (Fig. 1B) levels. Stimulation of the distal end of the sural, tibial, or peroneal nerve did not affect plasma bGH levels, but increases in pituitary bGH of 23 and 26% were seen with distal sural and peroneal nerve stimulation, respectively (Fig. 4A). Although these increases were significant compared with the experimental control, the pituitary bGH values for distal sural or peroneal nerve stimulation did not exceed the range [31.6 ± 4.9 (mean ± SD)] of control pituitary bGH values calculated across experiments. Furthermore, while none of the sural or distal nerve stimulations fell outside this range, all proximal stimulation values were well beyond these limits. Plasma iGH concentrations were not different from control levels after any of these distal nerve stimulation paradigms (Figs. 1B and 4B). However, plasma iGH levels were increased 139% after stimulation of the distal ends of the peroneal + tibial + sural nerves together (Fig. 3B). This may reflect the relatively large muscle mass stimulated, which could have induced an iGH response via metabolic mechanisms.

Other plasma hormones and metabolites. Table 1 shows plasma triiodothyronine, thyroxine, testosterone, corticosterone, glucose, lactate, and triglyceride concentrations after proximal or distal stimulation of the sural, tibial, or peroneal nerve. The levels of these hormones and metabolites were within normal ranges in control animals and showed no changes after any of the stimulation paradigms. These data indicate that there were no metabolic perturbations in these ani-
the present experiments was sufficient to excite low-threshold group I and some group II afferent fibers that originate from muscle spindles and Golgi tendon organs, thus providing strong evidence of a proprioceptive mechanism for regulation of bGH, but not iGH, release. Furthermore, these experiments indicate that the mechanism for eliciting bGH secretion is similar in fast (e.g., flexor or extensor) muscles and that the amount of fast-muscle mass associated with the stimulated nerve (i.e., number of low-threshold receptors activated) had no effect on the magnitude of the bGH response to stimulation.

Evidence that bGH is of pituitary origin. Several studies provide evidence that bGH is of pituitary origin. First, bGH concentrations are approximately fourfold higher in jugular than in cardiac plasma, and stimulation by insulin further increases jugular, but not cardiac, plasma bGH (10). Second, bGH is present in anterior pituitary extracts and has been measured in both the granular and cytosolic fractions of pituitary somatotrophs (11). Third, bGH is secreted by anterior pituitary cells in culture, and, more specifically, is released from cultures enriched in somatotrophs (19). Fourth, bGH is undetectable in plasma of hypophysectomized rats (see Ref. 9 and present results). Finally, bGH concentrations are inversely related in pituitary and plasma samples from proximal tibial, peroneal, and/or sural nerve-stimulated rats (Fig. 1–3A). To further address the question as to whether elevated blood bGH concentrations after proximal nerve stimulation were due to secretion from the pituitary, we estimated the increase in the blood, assuming a blood volume of 7% of body weight and an approximate bGH half-life of ~10 min (unpublished observations). Our estimations are consistent with the interpretation that the increase in bGH in the plasma can be accounted for by the observed decrease in pituitary bGH.

Differences between bGH and iGH. Several lines of evidence demonstrate that the tibial growth, as used in the present bioassay, is not a measure of iGH. First, tibial growth was not inhibited by systemic administration of antibodies engendered to 22-kDa pituitary GH, the antibodies used to measure iGH; this indicates that the influence of 22-kDa GH on the bioassay is minimal (9, 11, 15). Second, basilar levels of plasma iGH in the rat

Table 1. Plasma hormone and metabolite concentrations after electrical stimulation of proximal or distal end of severed sural, tibial, or peroneal nerve

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<thead>
<tr>
<th>Plasma Hormone and Metabolite Concentrations</th>
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<tr>
<td></td>
<td>Triiodothyronine, ng/dl</td>
</tr>
<tr>
<td>Control</td>
<td>52 ± 5</td>
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<tr>
<td>Proximal sural</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Proximal tibial</td>
<td>54 ± 6</td>
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<tr>
<td>Proximal peroneal</td>
<td>50 ± 5</td>
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<tr>
<td>Distal sural</td>
<td>47 ± 4</td>
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<tr>
<td>Distal tibial</td>
<td>50 ± 3</td>
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<tr>
<td>Distal peroneal</td>
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Values are means ± SE. None of the values is significantly different from control.
are ~10 ng/ml whereas basal bGH levels are equivalent to 500–1,000 ng/ml of iGH (11). We also demonstrated basal plasma iGH values of ~10 ng/ml, but it is interesting to note that the basal plasma bGH levels reported in this manuscript are ~2,000 ng/ml. The reason for the higher basal bGH levels in the present series of experiments is unclear, but it could be related to the anesthetic state and type of anesthesia. In the former study (11), the rats were lightly anesthetized with ether for a brief period; however, in the present experiments, rats were deeply anesthetized with pento-barbital sodium for ~30 min. Third, insulin-induced hypoglycemia, fasting, or exposure to cold reduce pituitary bGH content in rats by as much as 65% while increasing plasma bGH, but the same conditions do not change pituitary or plasma iGH concentrations (9, 10). Finally, other differences in responses of iGH and bGH to spaceflight are noted in the introduction (see Refs. 17–19).

Assay sensitivity and specificity. Our bioassay and immunoassay have different sensitivities and different endpoints. The bioassay has a sensitivity of 1 μg and is specific for GH, because no other pituitary-related hormones (e.g., thyroid hormones, testosterone) produce epiphyseal widening of the magnitude or in the dose-response fashion seen after administration of GH (13). Furthermore, the plasma and pituitary samples from our nerve-stimulated rats do not contain sufficient levels of these hormones to induce the amounts of tibial growth seen after injection into the bioassay rats (13; unpublished observations). Although we cannot absolutely exclude the possibility of hormone interactions within the assay animal, the plasma levels of the other hormones that might induce growth in the control and stimulated rats are similar and thus cannot account for the differences in tibial growth rates seen in the bioassay results. In addition, all pituitary-dependent hormones, including the insulin-like growth factors, should be at minimal concentrations in the rats used for bioassay, because they were hypophysectomized 2 wk before being used in the experiments.

Physiological significance of bGH. The physiological significance of bGH has yet to be fully determined. However, the fact that bGH promotes tibial epiphyseal widening in a dose-response fashion paralleling 22-kDa GH suggests that bGH may also be an effective therapeu- tic agent in growth-related abnormalities. Clinical situations have been described in which afferent input from fast skeletal muscles associated with movement and posture can play a role in increasing bGH release, but not iGH release, in the anesthetized rat. This specific mechanism of regulation of release of bGH, however, could be shared with regulatory factors other than the neural one identified by the present experiments. These results raise numerous questions related to the control mechanisms and the potential ramifications of prolonged bed rest, spaceflight, spinal cord injury, and neuromuscular maladies that minimize or eliminate sensory input to the endocrine system.

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