Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats

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Adams, Gregory R., and Samuel A. McCue. Localized infusion of IGF-I results in skeletal muscle hypertrophy in rats. J. Appl. Physiol. 81: 2509–2516, 1996. Insulin-like growth factor I (IGF-I) peptide levels have been shown to increase in overloaded skeletal muscles (G. R. Adams and F. Haddad. J. Appl. Physiol. 81: 2509–2516, 1996). In that study, the increase in IGF-I was found to precede measurable increases in muscle protein and was correlated with an increase in muscle DNA content. The present study was undertaken to test the hypothesis that direct IGF-I infusion would result in an increase in muscle DNA as well as in various measurements of muscle size. Either 0.9% saline or nonsystemic doses of IGF-I were infused directly into a non-weight-bearing muscle of rats, the tibialis anterior (TA), via a fenestrated catheter attached to a subcutaneous miniosmotic pump. Saline infusion had no effect on the mass, protein content, or DNA content of TA muscles. Local IGF-I infusion had no effect on body or heart weight. The absolute weight of the infused TA muscles was ~9% greater (P < 0.05) than that of the contralateral TA muscles. IGF-I infusion resulted in significant increases in the total protein and DNA content of TA muscles (P < 0.05). As a result of these coordinated changes, the DNA-to-protein ratio of the hypertrophied TA was similar to that of the contralateral muscles. These results suggest that IGF-I may be acting to directly stimulate processes such as protein synthesis and satellite cell proliferation, which result in skeletal muscle hypertrophy.

insulin-like growth factor 1; growth factors; somatomedin; muscle DNA content; fibroblast growth factor-2

INDIVIDUAL SKELETAL MUSCLES can respond to increased loading by adapting their size and contractile characteristics (8). In contrast to the generalized skeletal muscle growth seen during development, this process results in selective hypertrophy of the affected muscles. A common example of this selective form of adaptation is the hypertrophy evident after resistance training of specific muscle groups. A more extreme example would be the rat model of compensatory hypertrophy in which a single muscle can be targeted via the removal of all synergists and increase in mass by as much as 100% (e.g., Ref. 1).

The sensitivity of individual muscles to changes in loading state suggests that mechanisms are functioning at the level of the myofiber to promote adaptation. Whereas many aspects of skeletal muscle physiology have been extensively studied, neither the mechanisms that transduce mechanical load into cellular-level signals nor the components of myofiber signaling pathways that promote hypertrophy have been clearly delineated.

One obvious process that must occur during skeletal muscle hypertrophy is increased protein accumulation. In addition, it has recently been suggested (3, 14) that muscle hypertrophy is accompanied by increases in the number of myonuclei, such that the ratio between myofiber size and myonuclear number is maintained within some finite range. Current theory suggests that new myonuclei in hypertrophied skeletal muscle arise from the proliferation of satellite cells and the subsequent differentiation and fusion of some of the satellite cell progeny with existing myofibers (28, 29, 31). Experiments in which satellite cells have been prevented from contributing myonuclei, via prior γ-irradiation, have found that satellite cell proliferation is an obligatory process for the development of this type of compensatory muscle hypertrophy (23, 25, 26).

Insulin-like growth factor I (IGF-I) is thought to be a critical modulator of skeletal muscle growth as well as overall somatic growth during mammalian neonatal development (7, 15). IGF-I is also known to promote hypertrophylike responses in muscle cell lines (15). The purported ability of IGF-I to stimulate both anabolic and myogenic effects in vitro suggests that this growth factor is a promising candidate as a component of a cellular-level signaling system in skeletal muscle.

We have recently reported that compensatory hypertrophy of rat plantaris muscles is accompanied by increased IGF-I peptide production in the affected muscle (1). This increased IGF-I production coincides with increases in muscle DNA and precedes measurable increases in muscle protein. This result has led us to hypothesize that IGF-I may be acting as an autocrine and paracrine signal, thereby stimulating increased protein production in myofibers and the proliferation of satellite cells.

A number of studies have attempted to augment muscle adaptation to resistance training in humans by increasing circulating IGF-I by the infusion of IGF-I itself or of growth hormone (GH), thus indirectly increasing circulating IGF-I. These studies have generally found that increased circulating levels of IGF-I had no effect on the degree of muscle hypertrophy or other measured responses to resistance training (e.g., Refs. 35, 41). Analogous animal studies have generally found that an experimental increase in the circulating levels of IGF-I in generalized somatic growth that includes an increase in the weight of some muscles (6, 19, 40). However, the muscle growth seen was either proportional to, or somewhat less than, the generalized increase in body size. As a result, these experiments do not directly address the concept of locally mediated responses to loading and the role of IGF-I in that process.

In contrast to the results with increased systemic IGF-I, Coleman et al. (11) have recently reported that overexpression of IGF-I in the skeletal muscles of mice...
leads to significant hypertrophy that was confined to that tissue. Because of the nature of the transgenic model, these animals were exposed to increased IGF-I production throughout the developmental period of rapid growth. As a result, it is not clear that this model provides insights into the role of IGF-I in the hypertrophy response to repeated episodes of increased muscle loading in mature animals.

The experiments described in this paper were designed to investigate the responsiveness of individual skeletal muscles in adult rats to local increases in IGF-I. Our hypothesis was that local increases in IGF-I peptide would mimic the signaling processes thought to take place after an increase in the loading of a skeletal muscle. Our results show that direct local infusion of nonsystemic doses of IGF-I into the tibialis anterior (TA) muscles of adult rats resulted in muscle hypertrophy that included an increase in muscle DNA content.

METHODS

For the primary study, 35 female Sprague-Dawley rats, weighing 220 ± 2 g, were randomly assigned to three groups. The TA was chosen as a target for these experiments because this muscle is a plantar flexor of the foot and as such does not directly bear the animal's body weight. The TA muscles of rats from the first group were infused with 0.9% sterile saline solution for either 2 wk (n = 5) or 3 wk (n = 5). In the second group, TA infusion consisted of recombinant human IGF-I (Genentech) at 0.9 µg/day for 3 wk (n = 10). In a third group, 1.9 µg/day of IGF-I were infused into the TA for 2 wk (n = 5).

In two additional groups, five rats each were randomly assigned to receive local infusion of either 1.9 µg/day recombinant human GH (Bachem) or 1.5 µg/day of recombinant human basic fibroblast growth factor-2 (FGF-2; Calbiochem, San Diego, CA) for 2 wk.

Local Infusion Technique

Two IGF-I doses were selected to roughly span a range of systemic doses that had been reported to have growth-inducing effects in rats (e.g., Refs. 27, 37, 38). Each IGF-I infusion dose was arrived at by scaling a systemic dose in milligrams per kilogram to the expected weight of the TA muscles of rats in the selected body weight range. The lower dose of 0.9 µg/day would be equivalent to a systemic dose of 2.0 µg·kg⁻¹·day⁻¹, whereas the higher muscle dose (1.9 µg/day) would approximate a 4.0 µg·kg⁻¹·day⁻¹ systemic dose for rats of this size.

Infusion of saline or one of the growth factors was accomplished via a catheter attached to a miniosmotic pump (Alzet model 2002 [14 days] or 2004 [21 days], Alza). The miniosmotic pumps were filled under aseptic conditions, following the manufacturer's instructions. All solutions were sterilized via µStar syringe filters (Corning-Costar) during pump filling. Catheters consisted of ~12 cm of C-flex implantable tubing (OD 0.8  in., ID 0.02 in.) bonded to ~2.5 cm of Tygon tubing (OD 0.03 in., ID 0.01 in.; Cole Parmer). The C-flex tubing is very flexible and was used to provide strain relief to prevent excess stress on the muscle fascia. The Tygon tubing was fenestrated by using a microtipped soldering iron (Antex). For catheter implantation, the rats were anesthetized with ketamine and acepromazine, 80 and 2 mg/kg, respectively, and incisions were made in the skin overlying the TA muscle and on the back ~4 cm caudal from the neck. Two small cuts were made in the layers of fascia overlying the TA muscle using iris scissors.

One cut was near the proximal end of the muscle, whereas the other was distal, near the tendon. The Tygon portion of the catheter was tunneled under the fascia of the TA and secured in place by a single stitch (4–0 Ethicon) that passed through the fascia and around the catheter at both the entry and exit points. The C-flex portion of the catheter was tunneled under the skin to the back incision. The catheter was then filled with the same solution placed in the pumps (e.g., saline or growth factor), and the distal end (Tygon) of the catheter was closed by tying off with 2–0 suture. The C-flex end of the catheter was then mated with the osmotic pump that had been primed by preincubation in sterile saline at 37°C. The pump was then placed under the skin via the back incision, and both incisions were closed. At the termination of the infusion protocols, the osmotic pumps were removed, and any remaining infusate was aspirated via a syringe to verify that the pumps had functioned correctly.

Based on the specifications for the osmotic pumps, the total volume delivered per day was 10.8 ± 0.48 µl (~0.45 µl/h) in the 2-wk groups and 5.3 ± 0.24 µl (~0.22 µl/h) in the 3-wk groups. Based on the findings of Sreter and Woo (33), the volumes infused per hour would represent ~0.6 and ~1.3% of the extracellular space, respectively, in rat TA muscles of this size.

As a validation of the effectiveness of the IGF-I infusion, six rats were implanted with 14-day osmotic pumps delivering 1.9 µg of IGF-I per day. Five days after the implantation surgery, these rats were anesthetized, and plasma samples were collected from the left ventricle. The TA and extensor digitorum longus (EDL) muscles were then removed and processed as described below.

Tissue Collection

Infusion validation group. Both the contralateral and infused TA muscles were removed and cut in half along the long axis of the muscle, separating them into a deep and a superficial (nearest the catheter) halves, then quick frozen between blocks of dry ice. These muscles were stored at -80°C for later analysis.

Primary study groups. Fourteen days after the implantation surgeries, rats from the saline control and the growth factor experimental groups were killed via an overdose of pentobarbital sodium (120 mg/kg). The TA muscles of both the infused and contralateral leg were removed, weighed, and quick frozen between blocks of dry ice. These muscles were stored at -80°C for later analysis. At 21 days, 5 saline control rats and the 10 rats receiving the 0.9 µg/day IGF-I dose were killed, and TA muscles were collected as described above.

Assessment of Muscle Hypertrophy

Muscle mass determination. After dissection, left and right TA muscles were quickly weighed on an electronic scale before further processing. The left ventricle from each rat was dissected free from the atria, arteries, and right ventricle and weighed. Both EDL muscles were also removed and weighed.

Muscle and myofibrillar protein determination. Muscle protein was determined from whole muscle homogenates by using the bichromatic method (17). Total muscle protein was calculated from the product of the concentration and the wet weight of the muscle sample recorded at death. Total myofibrillar protein was determined as previously described (39) by using the method of Solaro et al. (32). Briefly, muscle samples were weighed and then homogenized in ~20 vol of an ice-cold solution containing (in mM) 250 sucrose, 100 KCl, and 5 EDTA. The homogenate was washed (suspended, then centrifuged at 1,000 g for 10 min) succes-
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the EDL muscles, which lie directly beneath the TA, were similar for both the infused and contralateral sides, suggesting that the infused IGF-I affected only the target muscle. Based on these same measurements, neither GH or FGF-2 infusion had any apparent systemic effects.

In the validation group that had received infusion of 1.9 µg/day of IGF-I, plasma IGF-I levels were similar to those found in control rats (e.g., infused 558 ± 74 vs. control 569 ± 43 ng/ml). The lack of systemic effect from these local infusion protocols is not surprising considering the doses used. As an example, the highest IGF-I dose infused in the present study was 1.9 µg/day. This contrasts with reports from the literature that generally employed systemic doses of IGF-I in the range of 200–300 µg/day (18, 24, 27, 37, 38).

TA Muscle Hypertrophy

The implantation and infusion protocol, in and of itself, did not result in alterations in muscle size. For example, at the end of 3 wk of treatment, the saline-infused TA muscle weights were 453 ± 7 mg, whereas the contralateral muscles weighed 453 ± 9 mg.

In contrast to the saline response, infusion of IGF-I at a dose of 0.9 µg/day for 3 wk or 1.9 µg/day for 2 wk resulted in ~9% increases in whole muscle mass, compared with either the contralateral TA muscles or with those of the saline-infused TA (Fig. 2). Similar increases in TA muscle weight are seen when the data are expressed normalized to body weight (P < 0.05) (data not shown).

The total muscle protein content (e.g., mg/muscle) of the IGF-I-infused muscles was increased relative to contralateral muscles (Fig. 3). The relative increase in total muscle protein was greater in the 1.9-µg-dose group after 2 wk (~47%) than in the 0.9-µg-dose group after 3 wk (~22%) of IGF-I infusion, suggesting that there was a dosage-dependent effect. Saline infusion had no effect on the total protein content of TA muscles (e.g., 3 wk saline-infused: 132 ± 6 mg; contralateral: 134 ± 6 mg). Local infusion of GH resulted in a significant increase (122 ± 5 vs. 145 ± 8 mg) in total muscle protein content, whereas FGF-2 infusion did not result in significant changes in this parameter.

Fig. 2. Effects of 0.9% saline or growth factor infusion on TA muscle total protein content. *P < 0.05 vs. contralateral; #P < 0.05 vs. saline.

TA Muscle DNA Content

As noted above, the local infusion protocol did not appear to result in equivalent distribution of growth factors to all regions of the muscle. As a result, the extrapolation of total muscle protein from the muscle samples taken near the infusion site might be expected to lead to an overestimation of these values. In addition, in the IGF-I-infused muscles, there appeared to be a trend toward an increase in the total protein and myofibrillar protein concentration. These two factors resulted in an apparently disproportionate increase in muscle protein compared with the muscle wet weight (cf. Fig. 3 vs. Fig. 2).

In each case, the myofibrillar protein concentration of the growth factor-infused muscles was similar to that of control or contralateral muscles (e.g., IGF-I infused 169 ± 3 vs. contralateral 172 ± 8 mg/g). This indicates that myofibrillar protein increased in parallel with the total protein and strongly suggests that the growth factor-induced increases reflect effects on the myofibers, as opposed to some nonmuscle cell type (e.g., fibroblasts).

As with muscle weights and total muscle protein, saline infusion did not affect the total DNA content of the infused TA muscles (Fig. 4). However, infusion of IGF-I, GH, or FGF-2 resulted in significant increases in TA muscle DNA content compared with either the
IGF-I-induced muscle hypertrophy

We previously reported that IGF-I peptide concentrations increased in muscles undergoing hypertrophy due to functional overload (1). Furthermore, the increases in muscle IGF-I peptide paralleled increases in IGF-I mRNA, suggesting that the increased IGF-I was of muscle origin. These results, in conjunction with an extensive body of literature regarding the hypertrophic and anabolic effects of IGF-I in vitro (15), lead us to speculate that this growth factor acts in an autocrine and/or paracrine mode to mediate the hypertrophy process in skeletal muscle. Accordingly, we designed the experiments described herein to determine whether exogenously supplied IGF-I, provided in doses that avoided generalized somatic growth, would induce muscle hypertrophy in adult rats in the absence of alterations in muscle loading. We chose the TA muscle as a target for these experiments because it is a non-weight-bearing muscle and has proven to be relatively insensitive to such interventions as decreased loading induced by the hindlimb-suspension model (2).

The results of this experiment demonstrate that IGF-I infusion stimulates hypertrophy of the target skeletal muscle. The various growth factor infusions had no apparent effects on nearby muscles or the heart or body weight of the rats. In particular, the heart and body weights of rats are known to be sensitive indicators of systemic effects of GH and IGF-I (13, 16, 24). The experimental design employed avoided inducing somatic growth via the delivery of low doses of IGF-I. The highest effective dose delivered in this study was ~7.9 µg·kg⁻¹·day⁻¹. In contrast, effective systemic doses found in the literature range from 1.0 to 6.9 mg·kg⁻¹·day⁻¹ (e.g., Refs. 13, 27, 37, 38).

The results of this study are qualitatively similar to those reported by Coleman et al. (11), who found that increased muscle IGF-I production resulted in significant muscle hypertrophy in transgenic mice. However, the degree of hypertrophy seen in transgenic mice was greater than that reported here. The design of the present study differs from the transgenic model in that the increased muscle IGF-I levels were initiated in adult rats and thus were not present during the prenatal or postnatal development period. This design would be expected to more directly mimic conditions found during muscle adaptation to increased loading in adult animals.

In addition to the increases in muscle mass and protein accumulation, IGF-I infusion stimulated an increase in DNA content (µg/muscle). This result is similar to that seen when a skeletal muscle hypertrophies in response to increased loading (1). The process of muscle hypertrophy is known to include an increase in the number of myonuclei (see Refs. 3, 28, 29, 31). Because mature mammalian myofibers are thought to be unable to reenter the cell cycle (9, 34), current thought is that increases in myonuclei result from the proliferation, differentiation, and fusion of satellite cells with existing myofibers (30).

The theory that satellite cells participate in the hypertrophy process is supported by a number of reports based on a variety of experimental models (3, 21, 23, 25, 26, 29). Of these studies, several indicate that the hypertrophy that occurs in response to increased loading requires mitotically competent satellite cells in order to proceed (23, 25, 26). These studies reported that the incapacitation of satellite cells via γ-irradiation actually prevented muscle hypertrophy from occurring in response to increased loading (23, 25, 26). The concept that the process of muscle hypertrophy requires a coordinate increase in muscle DNA is also supported by recent findings suggesting that there is a finite relationship between the size of a muscle fiber and the number of myonuclei present in that cell (3, 14).

Various studies have reported that IGF-I stimulates anabolic responses as well as cellular proliferation and differentiation in a number of systems used to study skeletal muscle (4, 15). The increase in DNA seen in the present study suggests that IGF-I may be coordinating the hypertrophy process, possibly by stimulating cellular proliferation, presumably in satellite cells, as well as an increase in protein production, thereby maintaining a proportionality between the size of the muscle and its DNA (myonuclear) content.

The data presented in this study are open to a variety of interpretations. For example, it is possible that satellite cell activation is stimulated by the hypertrophy process itself. In this scenario, IGF-I might be initially exerting anabolic effects that subsequently stimulate satellite cells via some other messengers such as hepatocyte growth factor or FGF-2 (5, 36). In this scheme, IGF-I would then be important for the stimulation of differentiation of these new satellite cells. Whereas such a process has yet to be elucidated, the maintenance of the DNA-to-protein ratio in conjunction with the proportional increase in myofibrillar protein seen in the present study clearly suggests that IGF-I is modulating these two processes during hypertrophy.

Fig. 5. Effects of infusion of 0.9% saline or growth factor infusion on DNA-to-protein ratio of rat TA muscles. *P < 0.05 vs. contralateral; #P < 0.05 vs. saline.
In addition to IGF-I, a variety of other growth factors such as GH and FGF-2 have been implicated in the control of muscle growth and/or hypertrophy. GH is generally thought to exert many of its growth-promoting effects primarily via stimulating IGF-I production by the liver and, to a much lesser extent, other tissues such as kidney and muscle. In this study, we found that GH infusion resulted in muscle hypertrophy and an increase in the IGF-I concentration. Thus it would seem reasonable to suggest that the hypertrophy seen with GH infusion resulted indirectly via the increase in IGF-I.

FGF-2 is known to have mitogenic effects in skeletal muscle cell lines (15). It is interesting to note that in this study infusion of FGF-2 stimulated a significant increase in muscle IGF-I concentration and total DNA but not in muscle protein content (Fig. 3). This result is in sharp contrast to that seen with increased IGF-I resulting from IGF-I or GH infusion, where increases in muscle DNA and protein appeared to be coordinated such that the DNA-to-protein ratio remained unchanged (Fig. 5). FGF-2 has been shown to stimulate IGF-I production by fibroblasts in vitro (10), which may account for the increase in muscle IGF-I. However, FGF-2 is known to be antagonistic to the differentiation of myogenic cells into myoblasts (15, 22) and thus may counter a critical step in the hypertrophy process. Therefore, the increase in DNA seen with FGF-2 may reflect the proliferation of satellite cells or some other cell type such as fibroblasts. The failure of FGF-2 infusion to result in hypertrophy is likely due to the lack of differentiation necessary to complete the process.

To date, the literature suggests that IGF-I is unique among the well-characterized growth factors in its ability to stimulate both the proliferation and differentiation in myogenic cells (15). In a previous study (1), we found that endogenous increases in IGF-I production by overloaded skeletal muscles precedes measurable hypertrophy. In the present study, we demonstrated that exogenous IGF-I could stimulate muscle hypertrophy. Taken together, the results of these two studies strongly suggest that IGF-I may be a key agent in the signaling pathways that allow individual skeletal muscles to adapt to increased loading. IGF-I appears to be able to stimulate and/or coordinate both the anabolic processes necessary to increase muscle protein and the recruitment of satellite cells, thereby providing new myonuclei and preserving the equilibrium between the number of myonuclei and the size of the myofibers.

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