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Role of IGF-I and IGF-binding proteins within diaphragm muscle in modulating the effects of nandrolone

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Lewis, Michael I., Gail D. Horvitz, David R. Clemmons, and Mario Fournier. Role of IGF-I and IGF-binding proteins within diaphragm muscle in modulating the effects of nandrolone. *Am J Physiol Endocrinol Metab* 282: E483–E490, 2002. First published October 23, 2001; 10.1152/ajpendo.00191.2001.—Recent studies suggest that the anabolic effects of testosterone in muscle may be mediated, in part, by the insulin-like growth factor (IGF) system. The aim of this study was to examine the effects of nandrolone (NAN) on both IGF-I and IGF-binding proteins (IGFBPs) in the diaphragm muscle of 1-yr-old female rats. NAN (6.6 mg·kg^{-1}·day^{-1}) was infused continuously for 17 days using a subcutaneous Silastic implant, whereas controls (CTL) received blank capsules. Muscle fibers were classified immunohistochemically, and fiber cross-sectional areas (CSA) were determined quantitatively. IGF-I levels in both serum and muscle were determined by RIA. Immunoreactivity to an IGF-I antibody was used to localize IGF-I expression within individual muscle fibers. Muscle IGFBPs were determined by SDS-PAGE and Western ligand blotting and measured by scanning densitometry. Body weight was higher in the NAN group compared with CTL (9.4 ± 4.5% vs. −0.6 ± 3.1%). There were no changes in the fiber composition of the diaphragm. NAN increased the CSA of type IIa (20%) and type IIx/b (30%) diaphragm fibers. Levels of IGF-I in the diaphragm muscle were significantly higher (50%) in NAN-treated animals. Immunohistochemistry revealed increased localization of IGF-I within type IIx/b diaphragm fibers. In addition, NAN increased IGFBP-3 within the diaphragm (69%), whereas IGFBP-4 decreased (40%). We conclude that NAN-induced diaphragm muscle fiber hypertrophy is mediated, in part, by influences of the IGF system within the muscle, such that coordinated changes in IGFBPs reflect a direction of change that has been associated with an anabolic response in other test systems.

anabolic steroids; autocrine/paracrine effects; insulin-like growth factor I; insulin-like growth factor-binding proteins; muscle fiber hypertrophy; respiratory muscles

IN RECENT YEARS, it has become clearly evident that anabolic steroids may exert distinct effects on the structure and function of skeletal muscles, including the respiratory muscles, in both animal (2, 43, 44, 48) and human models (1, 50). We recently reported that nandrolone administered to adult male hamsters produced significant hypertrophy of all diaphragm muscle fibers and improved isometric and isotonic contractile function (35). The degree to which type IIx diaphragm fibers hypertrophied after nandrolone administration was almost double that noted for types I and IIa fibers (35). Similarly, Bisschop et al. (2) reported selective hypertrophy of type IIx/b fibers of the diaphragm and gastrocnemius muscles of male and female rats given nandrolone decanoate.

Although the mechanisms underlying muscle fiber hypertrophy following the use of anabolic steroids are largely unknown, there is recent evidence that another growth-promoting agent, insulin-like growth factor I (IGF-I) may, at least in part, be implicated. For example, testosterone administration has been reported to increase serum IGF-I levels in normal men (26), whereas high endogenous serum levels of testosterone in hyperandrogenic females was associated with increased serum IGF-I (15). Much emphasis has been placed recently on the IGF system within muscle and other tissues in mediating local autocrine/paracrine effects (18, 36, 54). In this regard, Urban et al. (55) reported increased mRNA concentrations of IGF-I and reduced IGF-binding protein (IGFBP)-4 mRNA concentrations in vastus lateralis muscle biopsies from elderly men given testosterone, suggesting a distinct influence of the anabolic steroid on the intramuscular IGF-I system. Similarly, in rodent models, augmented tissue levels of IGF-I mRNA have been reported in the diaphragm (22), uterus (47), or mandibular condyle (37) after anabolic steroid administration. In contrast, reduced intramuscular IGF-I mRNA levels were noted in young men rendered testosterone deficient by the short-term administration of a gonadotropin-releasing hormone agonist (38).

The possible cellular mechanisms underlying respiratory muscle fiber hypertrophy with the use of anabolic steroids have not previously been explored. This is of interest, as anabolic steroids are currently being considered as therapy to improve respiratory muscle function in various depleted patient populations (for

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example, chronic obstructive pulmonary disease; 50). The present study thus addressed three specific aims regarding the impact of anabolic steroids on the intramuscular IGF system of the diaphragm, a major inspiratory muscle. These were *1*) to evaluate, in an animal model, the overall expression of IGF-I in the diaphragm after the administration of a potent synthetic anabolic steroid (nandrolone), *2*) to localize IGF-I expression within individual diaphragm fibers with regard to both specific fiber types and the relative degree of fiber hypertrophy, and *3*) to determine the overall expression of IGFBPs within the diaphragm muscle with the use of anabolic steroids, as the former are important regulators of IGF-I actions (31).

We thus examined two major hypotheses: *1*) that enhanced IGF-I expression would occur in those individual diaphragm fibers that hypertrophied after the use of nandrolone and *2*) that the expression of IGFBPs within the diaphragm following the use of anabolic steroids would reflect a direction of change that would be expected to facilitate IGF-I action intramuscularly.

METHODS

Animal Groups

Fourteen adult female Sprague-Dawley rats with initial body weights of ~282 g were studied and divided into two groups, control (CTL; $n = 7$) and nandrolone (NAN) treated ($n = 7$). All animals were housed in individual cages with the ambient temperature maintained at 22°C and the light cycle fixed at 12 h on and 12 h off. Water and food (Purina rat chow) were provided ad libitum to both groups. The experimental protocol was approved by the Animal Use and Care Committee of the Cedars-Sinai Medical Center/Burns and Allen Research Institute.

Anabolic Steroid Administration

A pure powder form of NAN (Steraloids, Newport, RI) was administered over a period of 17 days by use of a controlled-release Silastic capsule implanted subcutaneously under general anesthesia [ketamine 100 mg/kg and xylazine 10 mg/kg ip (57)]. Blank Silastic capsules were implanted into CTL animals. Although the release of the anabolic steroid was constant over the experimental period, the relative dose (i.e., normalized for body weight) decreased with body weight gain. Thus the initial dose of NAN administered was 6.6 ± 1.2 $mg \cdot kg^{-1} \cdot day^{-1}$, with the dose at the conclusion of the experimental period being 6.0 ± 0.9 $mg \cdot kg^{-1} \cdot day^{-1}$. We have reported on the efficacy of a similar Silastic delivery system primed with testosterone in male hamsters, which significantly raised serum levels of testosterone (13).

Immunohistochemical Studies

Under general anesthesia (pentobarbital sodium 6 mg/100 g body wt ip), the diaphragm was rapidly excised, and a segment of the midcostal region was mounted on cork at its determined resting length and rapidly frozen in isopentane (which had been cooled to its melting point by liquid nitrogen). The remaining portion of the diaphragm to be used for biochemical studies was rapidly frozen in liquid nitrogen and stored at −80°C until analysis. Serial cross sections of the diaphragm were cut at 10-μm thickness using a cryostat (model 2800 E, Reichert-Jung) kept at −20°C.

Identification of myosin heavy-chain isoforms and IGF-I. The identification of IGF-I and various myosin heavy-chain (MyHC) isoforms in the rat diaphragm was obtained by an indirect immunoperoxidase technique. Serial muscle cryosections were dried at room temperature, fixed in cold acetone for 5 min, washed with phosphate-buffered saline (PBS) for 5 min, and incubated in normal goat serum for 15 min at room temperature. Sections were incubated for 2 h at room temperature in one the following mouse anti-MyHC monoclonal antibodies (diluted in PBS): BA-D5 (1:10) reacting with MyHC-1; SC-71 (1:10) reacting with MyHC-2A; BF-F3 (1:10) reacting with MyHC-2B; and BF-35 (1:20) reacting with all MyHCs except MyHC-2X (Ref. 49; antibodies provided by Regeneron Pharmaceuticals, Tarrytown, NY). Additional sections were similarly incubated in rabbit polyclonal antiserum UB2–495 (1:10) specific for IGF-I [provided by the National Hormone and Pituitary Program (NHPP) of the National Institute of Diabetes and Digestive and Kidney Diseases]. Cross-reactivity with IGF-2 has been reported to be 1.5–1.9% (technical data provided by NHPP). Sections were rinsed with PBS and exposed to an appropriate biotinylated secondary antibody for 30 min at room temperature. Control sections were exposed to secondary antibodies only. Sections were rinsed with PBS and exposed to the avidin-biotinylated enzyme complex (ABC; Elite PK-6100, Vector) reagent for 20 min at room temperature. Sections were rinsed again with PBS, and visualization was obtained after exposure to the peroxidase substrate 3-amino-9-ethylcarbazole (AEC peroxidase substrate kit) for 10 min. Sections were washed for 5 min and mounted with glycerin jelly mounting medium. On the basis of differences in immunoreactivity for MyHC, muscle fibers were classified into several types, i.e., type I (positive for MyHC-1), type IIa (positive for MyHC-2A), type IIc (positive for both MyHC-1 and MyHC-2A), and type IIx/b (positive for MyHC-2B or negative for all MyHCs except MyHC-2X). In each muscle, fiber type proportions were determined from a sample of ≥300 fibers, which were selected from various fields within the entire cross section and were representative of the entire profile of the segment.

Fiber cross-sectional areas. Muscle fiber cross-sectional area (CSA) was determined from microscopic images of digitized muscle sections with the use of a computer-based imaging processing system. The latter is composed of a Leitz Laborlux S (Leica) microscope, CCD video camera system (model VI-470; Optronics Engineering, Goleta, CA), high-resolution Trinitron color video monitor (model PVM-1343MD, Sony), 486 DX-50 MHz personal computer with a Targa$^+$ imaging board (Truevision), and Mocha image analysis software (version 1.20; Jandel, San Rafael, CA). A microscope stage micrometer was used to calibrate the imaging system for morphometry. The CSA of 200–300 individual fibers (i.e., sampled from those used in the analysis of fiber proportions) was determined from the number of pixels within manually outlined fiber boundaries.

Biochemical Studies: IGF-I

Serum IGF-I. Serum total IGF-I concentration was determined by RIA from blood samples obtained at the terminal experiments. Before RIA, IGF-I was extracted from the serum, and IGFBPs were precipitated by incubation in acid-ethanol (10). Supernatants were neutralized, and the RIA (33) was performed using a commercial kit specific for rodent IGF-I (DSL-2900; Diagnostic Systems Laboratories, Webster, TX) according to the manufacturer's protocol. This assay shows high cross-reactivity for hamster, mouse, and rat antigens (33). The intra-assay coefficient of variation is 5.9%

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and the interassay coefficient of variation is 9.7%. The sensitivity of the assay allows the detection of IGF-I peptide levels of >21 ng/ml.

Diaphragm muscle IGF-I. Frozen diaphragm tissue was pulverized in liquid nitrogen, IGF-I was extracted twice in acetic acid (1 mg/10 μl), and 100-μl aliquots of the supernatant were lyophilized and stored frozen overnight (10). Aliquots were resuspended in assay buffer and assayed using the same RIA kit, as described in *Serum IGF-I*, for the determination of muscle IGF-I.

Biochemical Studies: Diaphragm Muscle IGFBPs

For protein extraction, frozen muscle samples were pulverized at −80°C, transferred to a lysis buffer containing Tris·HCl, EDTA, and 2% Triton X-100, mixed, sonicated for 20 s, and centrifuged for 5 min at 12,000 rpm at 4°C, and the supernatant was collected and protein concentration determined. For the identification and quantitation of IGFBPs (53), protein extract samples were electrophoresed by 12.5% SDS-PAGE at 20 mA for 1.5 h and electrotransferred onto Immobilon (Millipore) membranes at 70 mA for 1 h (28). The filters were probed for IGFBPs by ligand blotting using ^{125}I-labeled IGF-I. ^{125}I-IGF-I had a specific activity of 230 μCi/μg. IGFBPs were visualized by autoradiography. Molecular weights were estimated from prestained standards in adjacent lanes. The autoradiograms were aligned with a blot and used as a template to mark the position of the IGFBP bands. Individual bands were scanned in duplicate and quantitated by densitometry.

Biochemical Studies: Serum Testosterone

Because quantitative NAN levels in rats are not available, serum testosterone levels were measured as a "surrogate" for NAN influences. Serum testosterone levels were measured after extraction with ethyl acetate and hexane by specific RIA with the use of reagents from ICN (Costa Mesa, CA), as previously described by Wang et al. (58). Because there is known cross-reactivity of the antiserum used in the testosterone RIA with several different anabolic steroids of similar structure to testosterone (58), we determined the degree of cross-reactivity for NAN. This was done by testing a large range of NAN concentrations with the same assay and comparing them with testosterone standards.

Statistical Analysis

Statistical analysis for muscle fiber type and CSA was performed using a one-way ANOVA with the experimental factor being the administration of NAN. A Newman-Keuls test was performed post hoc to compare differences in independent groups and to determine overall significance with an α-level of 0.05. Analysis of biochemical parameters was done using Student's *t*-test. A P value of <0.05 was regarded as significant. All data are presented as means ± SE.

RESULTS

Animal Body Weights

The body weights of CTL and NAN animals were similar before the start of the experimental period (Fig. 1). After 17 days of exposure to the anabolic steroid, the body weights of NAN animals increased significantly (9.4 ± 4.5%; $P < 0.001$), whereas those of CTL rats remained unchanged (−0.6 ± 3.1%; Fig. 1).

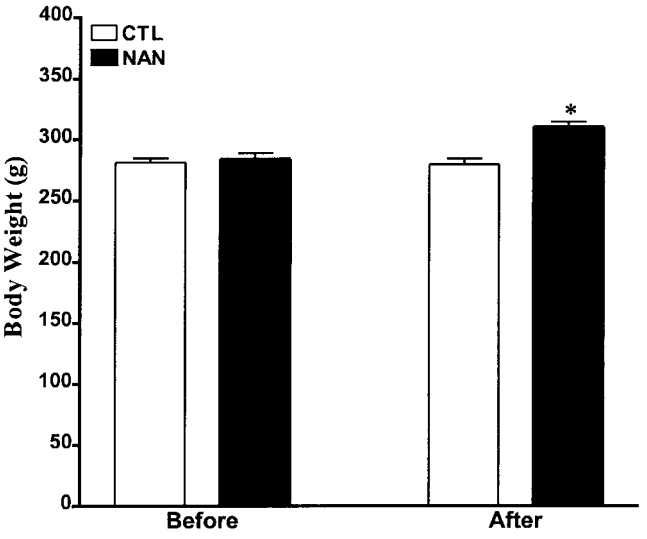

Fig. 1. Initial and final body weight of control (CTL) and nandrolone (NAN)-treated animals. There was a significant increase in body weight after NAN therapy (9.4%; *$P < 0.001$). Values are means ± SE.

Muscle Fiber Type Proportions

No alteration in fiber type proportions was observed in the diaphragm with the administration of NAN (Fig. 2*A*).

Muscle Fiber CSA

In the diaphragm, significant differences between groups in the CSA of type IIa (20%; $P < 0.05$) and type IIx/b (30%; $P < 0.01$) fibers were observed (Fig. 2*B*).

Biochemical Studies: IGF-I

Serum IGF-I. No differences were observed between the groups with regard to serum IGF-I levels (CTL: 287.6 ± 106.1; NAN: 297.5 ± 86.0 ng/ml).

Muscle IGF-I. The levels of IGF-I within the diaphragm muscle were significantly elevated in the NAN animals compared with CTL (50%; $P < 0.05$; Fig. 3).

Muscle Fiber IGF-I Immunoreactivity

Photomicrographs of diaphragm sections that reacted with IGF-I antibodies from representative CTL and NAN-treated animals are depicted in Fig. 4. A difference in IGF-I immunoreactive staining of diaphragm fibers is evident in the NAN-treated compared with CTL animals. The mean gray-level intensities of IGF-I immunoreactivity within individual fibers of the diaphragm were determined to better localize the upregulation of IGF-I within specific cell types and to determine whether the localization corresponded to fibers exhibiting a hypertrophic response. In the diaphragm, levels of IGF-I intensity were significantly higher in type IIx/b fibers of NAN animals compared with CTL (29%; $P < 0.01$). This correlates well with the hypertrophy of type IIx/b diaphragm fibers in these animals.

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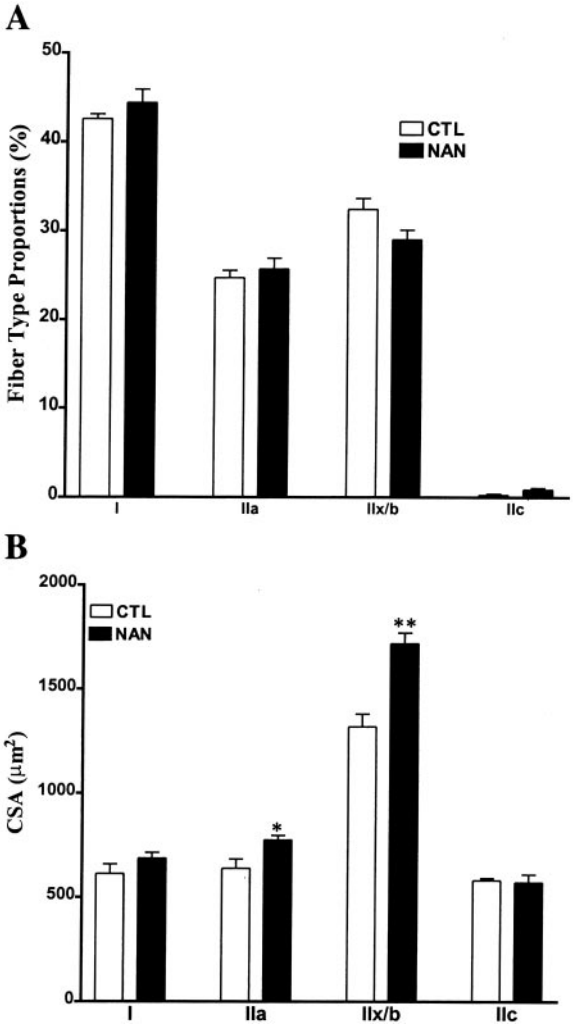

Fig. 2. Diaphragm muscle fiber proportions (*A*) and individual fiber cross-sectional area (CSA; *B*) in CTL and NAN-treated animals. Although there were no changes in diaphragm fiber proportions, there was a significant increase in the mean CSA of type IIa (20%; $^*P < 0.05$) and type IIx/b (30%; $^{**}P < 0.01$) fibers after NAN therapy. Values are means ± SE.

IGFPBs

Analysis of the diaphragm extracts from the NAN animals showed that the mean IGFBP-3 level was significantly higher (69% by scanning densitometry; $P < 0.01$; Fig. 5), whereas the mean IGFBP-4 level was significantly lower (40% by scanning densitometry; $P < 0.01$; Fig. 5).

Serum Testosterone

Serum testosterone level in CTL rats was 0.44 ± 0.23 ng/ml. This is within the range reported in the literature for female rats, which varies with the estrous cycle (0.1 to 0.6 ng/ml; Ref. 46). The testosterone level in NAN animals was 3.70 ± 1.18 ng/ml. Although one might expect very low endogenous testosterone values due to suppression by NAN, the higher levels are accounted for by an extremely large degree of cross-reactivity of NAN for the testosterone RIA antiserum. Our cross-reactivity studies revealed this to be 11.5%. The data thus support pharmacological dosing of NAN by our constant release method.

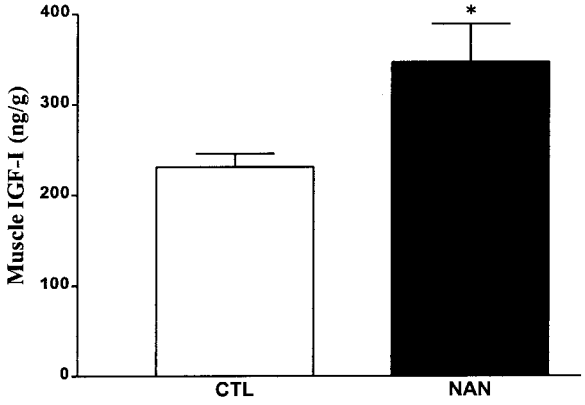

Fig. 3. Diaphragm muscle insulin-like growth factor I (IGF-I) concentration (ng/g) determined by RIA in CTL and NAN-treated animals. There was a significant increase in IGF-I level after NAN therapy (50%; $^*P < 0.05$). Values are means ± SE.

DISCUSSION

This study demonstrated a significant increase in body weight with the administration of NAN over a

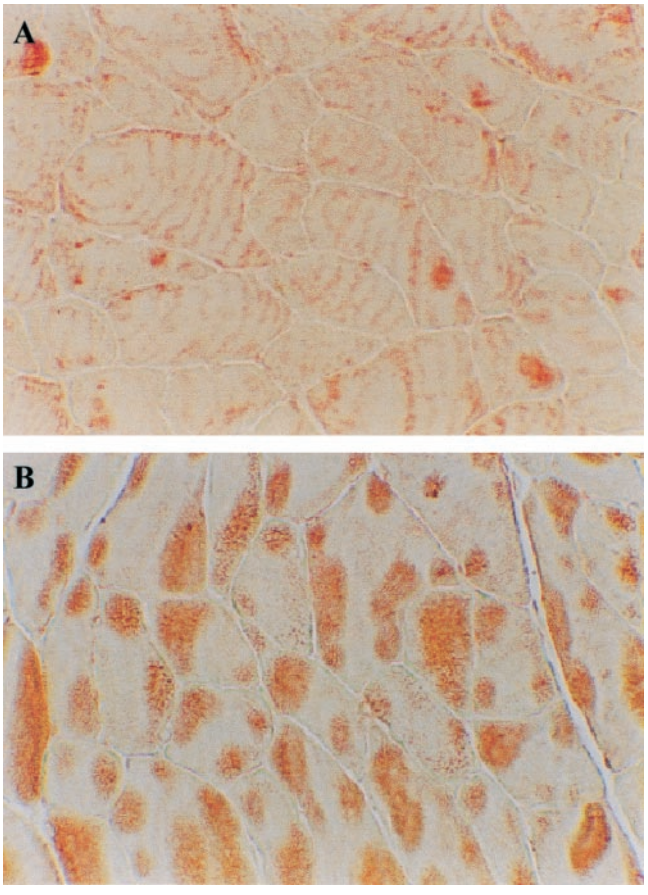

Fig. 4. Photomicrographs depicting IGF-I immunoreactivity in diaphragm fibers of CTL (*A*) and NAN-treated (*B*) animals. Note an increase in immunoreactive staining within fibers after nandrolone therapy.

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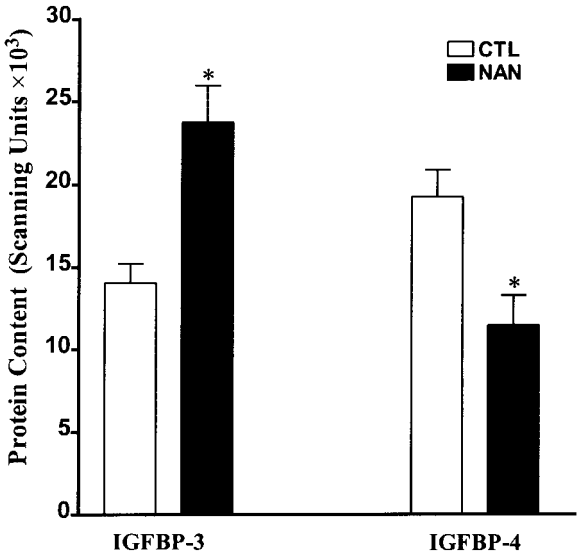

Fig. 5. Mean levels (protein content) of IGF-binding protein (IGFBP)-3 and IGFBP-4 in the diaphragm of CTL and NAN-treated animals. There was a significant increase in the amount of IGFBP-3 (69%; $*P < 0.01$) and significant decrease in the amount of IGFBP-4 (40%; $*P < 0.01$) of the diaphragm muscle after NAN therapy. Values are means ± SE.

17-day period. NAN was associated with a significant increase in the CSA of diaphragm type IIa and type IIx/b fibers. The overall expression of IGF-I within the diaphragm muscle was increased after the administration of the anabolic steroid. Localization of the IGF-I response within the diaphragm by immunohistochemical techniques revealed significantly enhanced IGF-I immunoreactivity within the most hypertrophied type IIx/b fibers. Furthermore, administration of NAN was also associated with significantly increased levels of IGFBP-3 and decreased levels of IGFBP-4 in the diaphragm muscle.

Muscle IGF-I

In the present study, the administration of a synthetic anabolic steroid resulted in significantly higher levels of muscle IGF-I. In recent years, it has become increasingly apparent that autocrine and paracrine actions of the IGF system within skeletal muscle may play a major role not only in myogenesis but also in the maintenance of muscle fiber growth or integrity in the postnatal state (18, 20, 31). Thus alterations in the local IGF milieu within muscle might be expected to exhibit distinct effects on muscle protein turnover and, therefore, its constituent muscle fibers. IGF-I has been demonstrated to stimulate protein synthesis in human, animal, and in vitro models (21, 27, 31, 32, 45). The enhanced protein synthesis, as demonstrated by the hypertrophic effect on diaphragm muscle fibers, likely reflects direct local autocrine influences of IGF-I within muscle fibers as well as indirect paracrine stimulatory effects of secreted IGF-I on satellite cells (4). In addition, unlike growth hormone, IGF-I may diminish protein degradation, possibly by inhibitory effects on the ubiquitin-proteasome system, which is a major pathway mediating muscle proteolysis (17, 27, 59). In this regard, IGF-I has been demonstrated to reduce mRNA stability of E2–14k, a key ubiquitin-conjugating enzyme (59). Increased IGF-I expression might thus be expected to exert complex influences on muscle protein turnover.

Anabolic steroids have been demonstrated to increase muscle IGF-I mRNA in human and animal studies. Urban et al. (55) reported increased IGF-I mRNA in the vastus lateralis muscle of elderly men in whom testosterone was administered. In addition, a combination of trenbolone acetate and estradiol resulted in increased IGF-I mRNA in the latissimus muscle of lambs (30). Recently, increments in rat diaphragm muscle IGF-I mRNA were reported after nandrolone decanoate administration over a 5-wk period (22). Our study extends these observations and further demonstrates increased expression of the protein within the muscle after NAN treatment. In the present study, serum IGF-I levels were not elevated with the use of the anabolic steroid as reported in some studies (15, 26). This suggests a locally mediated muscle effect of NAN on the IGF system within the diaphragm. Similarly, in the clinical study by Urban et al., serum IGF-I levels were also not elevated in the men receiving testosterone despite augmented IGF-I mRNA in the vastus lateralis muscle of these subjects. Although one might expect that increased serum levels of IGF-I could augment overall protein synthesis and thus increase local muscle expression of IGF-I, we have recently reported that long-term systemic infusion of IGF-I downregulates IGF-I protein expression in the diaphragm muscle, possibly due to a negative feedback effect (19, 34). Thus the results of the current study suggest an important effect of NAN therapy on muscle IGF-I, which may then result in autocrine/paracrine stimulation, an influence likely facilitated by muscle IGFBPs.

Muscle IGFBPs

At least six different IGFBPs have been well characterized and have important carrier functions and modulating influences on the IGF system (31). Most circulating IGF-I is bound to a 150-kDa complex comprising IGF-I, IGFBP-3, and an acid labile subunit (31). In recent years, it has been increasingly appreciated that IGFBPs may interact with and modulate not only the endocrine actions of IGF-I but also its local autocrine/paracrine influences in a variety of tissues, including skeletal muscle (8, 31, 40). For example, it has been shown that a variety of IGFBPs are expressed in a number of different muscle cell lines (18, 24, 29) as well as in the skeletal muscles of rodents, chicks, and larger animals (5, 23, 42, 51). Although the precise actions of IGFBPs expressed at the tissue level are unknown, it has been postulated that they act to modulate local IGF-I action by augmenting local IGF-I bioavailability and/or providing an IGF-I depot (6, 31, 40). In addition, IGFBPs may exert effects independent of the IGF system (56).

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In the present study, NAN administration resulted in increased expression of IGFBP-3 and reduced expression of IGFBP-4 in the diaphragm of treated rats. Although the IGFBPs may, under certain conditions, function to either facilitate or inhibit IGF-I functions (31), IGFBP-4 is the only binding protein thought to function only as an inhibitor of IGF-I (16, 31). In contrast, IGFBP-3 has been shown to both inhibit and enhance IGF-I actions in in vitro test systems (6, 31). Importantly, coincubation of IGFBP-3 with IGF-I stimulates aminoisobutyric acid uptake by cultured fibroblasts (9). Similarly, administration of other anabolic agents such as growth hormone has been shown to result in increased IGFBP-3 and decreased IGFBP-4 (7). Thus the increments of muscle IGFBP-3 and decrements in IGFBP-4 noted in the present report may be interpreted as a series of coordinated changes in the muscles to liberate more free IGF-I to bind its receptors. Of interest, IGFBP-4 mRNA levels were also reduced in vastus lateralis muscle biopsy specimens from elderly men receiving testosterone (55). Although the biochemical mechanisms responsible for alterations in muscle IGFBP expression are unknown, provocative data in androgen-sensitive human fibroblast cultures demonstrated reduced IGFBP-4 mRNA and protein in response to IGF-I. Furthermore, an increase in IGFBP-3 was noted in the culture medium after either IGF-I or testosterone treatment, with an additive effect noted with the combination of IGF-I and testosterone (60).

Fiber CSA

In the present study, significant hypertrophy of type IIa and type II x/b (IIx/b > IIa) fibers in the diaphragm was evident in NAN-treated rats. This was associated with increased muscle IGF-I expression in general, whereas immunohistochemical studies tended to localize the enhanced IGF-I expression to those most hypertrophied type II x/b diaphragm fibers. This strongly suggests that the anabolic steroid tended to exert its impact on muscle fiber size, at least in part, due to influences on the IGF system within the muscle. Furthermore, the changes in IGFBP expression highlighted earlier suggest a complex series of changes that result in facilitation of IGF-I actions. This greater IGF-I signaling would thus be expected to contribute to protein anabolism and fiber hypertrophy. It has been proposed that IGF-I may induce muscle fiber hypertrophy through signaling involving the Ca^{2+}-calmodulin-dependent protein phosphatase calcineurin pathways (41). The increased local IGF-I concentration in the muscle may also be accompanied by satellite cell activation to maintain the myonuclear domain size (i.e., volume of myoplasm per myonucleus) of individual muscle fibers. IGF-I has been shown to promote proliferation and differentiation of satellite cells and fusion of new myoblasts to promote hypertrophy (4, 14, 39). The concept is that each myonucleus regulates protein expression within a given myoplasmic volume, so that any increase in muscle fiber size would be required to be supported by new, additional myonuclei, which are provided by muscle stem cells (i.e., satellite cells; see Refs. 4, 39). Furthermore, increased local muscle protein accretion could be augmented by reduced protein degradation mediated by enhanced intramuscular concentrations of IGF-I.

The greatest degree of hypertrophy noted in type IIx/b diaphragm fibers with the use of NAN poses some interesting questions. In general, these fast-fiber types are not usually active under resting conditions and belong to diaphragm motor units that would be expected to be recruited only under conditions of increased mechanical or chemical demands (25, 52). The mechanisms underlying this selective hypertrophy and IGF system changes are unclear. Although recent reports have suggested that steroid receptors in "fast-twitch" muscles (e.g., extensor digitorum longus) exhibit a higher affinity than those in "slow-twitch" muscles (e.g., soleus), it is unclear whether this pertains to specific subtypes of fast-twitch (type II) fibers (3). Furthermore, there are no data on the concentrations of androgen, growth hormone, or IGF-I receptors in skeletal muscle fiber subtypes. Thus the precise mechanisms responsible for selective fiber type effects, at both cellular and biochemical levels, are unknown.

In summary, the present study demonstrates that the administration of anabolic steroids results in hypertrophy of diaphragm muscle fibers and that the specific fiber hypertrophy noted is likely mediated, at least in part, by increments in IGF-I within those specific fiber types. Furthermore, coordinated changes in the expression of IGFBPs within the diaphragm muscle reflect a direction of change that would be expected to facilitate autocrine/paracrine effects of IGF-I within the muscle.

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