Testosterone Mediates Satellite Cell Activation in Denervated Rat Levator Ani Muscle

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ABSTRACT

Denervation stimulates quiescent satellite cells in skeletal muscle to reenter the cell cycle. In the androgen-sensitive rat levator ani muscle (LA), this mitotic response to loss of neural input fails to occur in castrated animals. To elucidate the role of androgens in denervation-induced satellite cell proliferation, the denervated LA of castrated rats (Group A) was compared with that of animals infixed with testosterone implants after castration (Group B). Mean myofiber cross-sectional areas (Group A: 362.95 μm^2 ± 27.74; Group B: 403.13 μm^2 ± 53.87) and linear nuclear densities (Group A: 74.07 mm^{-1} ± 17.58; Group B: 104.13 mm^{-1} ± 4.06) were similar ($P > 0.05$) in both groups. The androgen-deprived myofibers of Group A, however, had a significantly lower nuclear content (271.0 ± 74.91 vs. 1,285.80 ± 81.74 in Group B; $P < 0.05$) on account of their considerably shorter mean length (3.44 mm ± 0.29 vs. 12.31 mm ± 0.92 in Group B; $P < 0.05$). The proportional representation of satellite cells in hormone-replaced, denervated muscle was more than twice that in the untreated group (Group B: 5.15 ± 0.83% vs. Group A: 2.28 ± 0.23%; $P < 0.05$). In absolute terms, the satellite cell number in Group B was approximately an order of magnitude greater than in Group A (408.4 × 10^3 vs. 38.08 × 10^3). The results confirm the absence of testosterone as the factor responsible for the inability of satellite cells in the LA of castrated rats to respond mitotically to the withdrawal of neural input after denervation. Anat Rec 263:19–24, 2001. © 2001 Wiley-Liss, Inc.

Key words: rat; levator ani; satellite cells; denervation; castration; testosterone

Satellite cells are a quiescent precursor component of skeletal muscle (Mauro, 1961). They are juxtaposed sublaminally to myofibers and represent the biological reserve upon which skeletal muscle draws for self-restoration (Carlson, 1973). Appropriate stimuli will cause these cells to proliferate and fuse with either the related myofiber or one another. One such stimulus is denervation and severance of the pudendal nerve has been shown to result in a twofold augmentation in the proportional representation of satellite cells in the levator ani muscle of the rat (Nnodim, 1999). A similar observation has been documented for limb muscles (Lu et al., 1997).

The levator ani muscle in the rat is very androgen-sensitive (Wainman and Shipounoff, 1941). In a recent study (Nnodim, 1999), the mean myofiber cross-sectional area was observed to diminish by approximately 50% two months post-castration. Unlike denervation, however, castration had no demonstrable effect on the satellite cell population. Also, denervation failed to elicit a proliferative response from satellite cells in the levator ani muscle of castrated rats.

The administration of exogenous testosterone to castrated adult rats is known to restore myofiber girth in the levator ani muscle (Venable, 1966; Gori et al., 1969) but a

mitogenic effect on satellite cells has not been reported. The lack of satellite cell activity after denervation in neutered rats however, suggests a role for androgens in satellite cell dynamics in the levator ani muscle. The present study tests the hypothesis that testosterone mediates the proliferative stimulus of denervation in the levator ani muscle of the rat.

MATERIALS AND METHODS

Two groups (A and B) of 4-month-old male rats of the WI/HicksCar strain (six per group) were used. All the rats were castrated and their levator ani muscle (LA) was denervated, using the surgical technique described in a previous report (Nnodim, 1999). The duration of the experiment was eight weeks.

Testosterone Replacement

Each rat in Group B received replacement testosterone via a 2-cm long implant placed in a subcutaneous pouch in the ventral abdominal wall on one side of the midline. Midway through the experiment, the implant was removed and a fresh one inserted on the contralateral side, as a precaution against the possibility of a local tissue reaction that might interfere with the diffusion of the drug.

The implants were made from silastic laboratory tubing (0.32 cm outer diameter, 0.17 cm inner diameter; Dow-Corning Corp., Midland, MI) charged with testosterone propionate (Sigma Co., St. Louis, MO). Both ends of the tubing were plugged with a small piece of the wooden handle of a cotton-tipped applicator and sealed with clear RTV silicone adhesive (Loctite Corp., Rocky Hill, CT). Before use, the implants were immersed in 95% ethanol overnight, followed by 24 hr in double-distilled water.

At the end of the experiment eight weeks later, venous blood samples were drawn from a random sample of three animals per group for testosterone estimation by competitive immunoassay, using automated direct chemiluminescence technology (ACS:180; Chiron Diagnostics, East Walpole, MA). The LA was then dissected out and processed for fluorescence microscopic analysis of dissociated muscle fibers as well as light and electron microscopy.

Tissue Processing and Microscopy

The LA was incubated in a 0.45% solution of collagenase type 3 (Worthington, Freehold, NJ) in phosphate-buffered saline (PBS; pH 7.4) for 75 min at 37°C to effect connective tissue digestion. Single fibers were then isolated by micro-manipulation and mounted on glass slides in Vectashield (Vector Labs., Burlingame, CA), a propidium iodide-incorporating medium. Nuclei were enumerated in five single fibers per muscle (and three muscles per group).

Myofiber cross-sectional areas were determined by computer-assisted planimetry of profiles in 1.5 μm-thick resin sections and satellite cells were directly counted by electron microscopy. These techniques have been fully described in two previous studies (Nnodim, 1999, 2000).

Animal Care

Conditions of animal care were fully in compliance with National Institutes of Health (NIH) guidelines. All surgical procedures were performed under ether inhalation anesthesia. The rats were given oral tetracycline (dissolved in their drinking water) postoperatively for one week. Terminally, euthanasia was by anesthetic overdose, followed by cervical dislocation.

Statistics

Comparisons of means were made using Student's t-test, with statistical significance set at $P < 0.05$. The relationship between the data obtained in the present study and those from normal rats (gonad- and nerve-intact) in an earlier investigation (Nnodim, 1999) was similarly evaluated.

RESULTS

Hormone Levels

One of the rats in Group A (androgen-deprived) had a terminal plasma testosterone level of 0.06 ng $\cdot$ ml^{-1}. The samples from the other two rats in the same group read "below check" (i.e., below the sensitivity threshold of the equipment).

In Group B, the mean level of plasma testosterone was 3.11 ± 0.51 ng $\cdot$ ml^{-1}.

Myofiber Cross-Sectional Area

Table 1 depicts the mean cross-sectional area of myofibers in the denervated LA of the castrated and untreated rats (Group A: 362.95 μm^2 $\pm$ 27.74) and that of their counterparts fitted with testosterone implants (Group B: 403.13 μm^2 $\pm$ 53.87). The difference between the two groups was statistically non-significant ($P > 0.05$).

Myofiber Length and Nuclear Count

The mean myofiber length in the denervated LA of castrated, testosterone-implanted rats (Group B) remained relatively unchanged (12.31 ± 0.92 mm; compared to 10.99 ± 0.54 mm of normal rats). In contrast, the myofibers in the denervated muscle of castrated, untreated rats (Group A) were reduced to about a third of normal length (3.44 ± 0.29 mm; $P < 0.05$).

The linear nuclear density of myofibers in the denervated LA of androgen-deprived rats (Group A) was 74.07 ± 17.58 mm^{-1}, compared to 104.13 ± 4.06 mm^{-1} for their hormone-replaced counterparts ($P > 0.05$; Table 2).

Individual myofibers in the LA of Group B rats contained approximately four times as many nuclei as those in the muscle of Group A rats ($1,285.80 \pm 81.74$ and 271.0 ± 74.9 respectively; $P < 0.05$; Table 3).

Satellite Cell Count

The satellite cell populations, as a percentage of the total number of sublaminal nuclei in the muscle, were $2.28 \pm 0.23\%$ in the untreated rats (Group A) and $5.15 \pm 0.83\%$ in the treated rats (Group B; Table 3).

TABLE 1. Myofiber cross-sectional areas*

Animal group	Area (μm^2)
Normal (gonad- and nerve-intact)	1,1519.98 ± 79.41
A (denervated and castrated)	362.95 ± 27.74
B (denervated, castrated, treated)	403.13 ± 53.87

*Values given as mean ± standard error. Statistically significant intergroup differences ($P < 0.05$): Normal vs. A; Normal vs. B. A vs. B statistically non-significant, $P > 0.05$.

TABLE 2. Myofiber nuclear density*

Animal group	Nuclear density (mm^{-1})
Normal (gonad- and nerve-intact)	79.58 ± 3.5
A (denervated and castrated)	74.07 ± 17.58
B (denervated, castrated, treated)	104.13 ± 4.06

*Myonuclei + Satellite cell nuclei per millimeter of myofiber. Values given as mean ± standard error. Fifteen myofibers analyzed per group (n = 3). Intergroup differences not statistically significant $P > 0.05$.

Table 3 also shows the derivation of whole-muscle satellite cell content from total nuclear and satellite cell frequency data, assuming a stable myofiber population (Venable, 1966; Viguie et al., 1997). The satellite cell number computed for the muscle of hormone-replaced rats (Group B) was approximately an order of magnitude greater than that of the castrated rats in Group A (408.4 × 10^3 vs. 38.08 × 10^3).

DISCUSSION

The present investigation is a sequel to a previous study of the individual and combined effects of denervation and androgen deprivation on the levator ani muscle of the rat (Nnodim, 1999). The earlier study showed that denervation alone caused an increase in the satellite cell population of the muscle in gonad-intact rats, but not after castration. That result suggested a need for further dissection of the interplay between neural and endocrine influences in the muscle.

Although all the rats used were castrated and had their LA denervated, those in Group B were infixed with testosterone implants. By this design, the LA of Group B rats was, in effect, only denervated and at no stage androgen-deprived. The question then was whether the denervated LA of castrated rats would become able to enrich its complement of satellite cells when provided with exogenous testosterone. In gonad-intact animals, the LA receives a fluctuating physiologic supply of hormone that contrasts with the steady diffusion from an implanted source. This lack of rhythmicity might have implications for the biological action of the hormone, possibly accounting for the fact that myofiber shortening did not occur in the dener-vated muscle of rats given exogenous testosterone as discussed below.

Notable similarities were apparent, however, between the LA of Group B rats and that of denervated, gonad-intact rats (Nnodim, 1999). Very pronounced myofiber atrophy occurred in both cases but to a degree not statistically significant from that induced by denervation and castration together. In addition, myofibrillar architecture was far less disrupted than in denervated and hormone-deprived muscle (Fig. 1). This observation suggests a role for testosterone in the maintenance of the internal structural organization of myofibers. Although denervation alone has been reported to deplete muscle disproportionately of contractile proteins (Fischer and Ramsey, 1946; Bass et al., 1969), it would seem that the additional loss of androgenic input is needed to bring about a collapse in the internal organization of the myofiber internum.

In the present study, the mean myofiber length in the denervated, hormone-deprived muscle (Group A) was reduced to about a third of control levels (3.44 ± 0.29 mm vs. 10.99 ± 0.54 mm). A similar regression in length was noted in the muscle of similarly treated rats in the preceding study (Nnodim, 1999) as well as after denervation or castration alone. This linear reduction would seem to be effected through the partial deletion of myofiber substance. Gori et al. (1967) described deep invaginations of the sarcolemma and basal lamina into the sarcoplasm as well as completely detached fragments of myofiber in the interstitium of LA during atrophy. They also noted that these "blocks of sarcoplasm" were subsequently phagocytosed by macrophages. Cellular deletion, both in part and in toto, in a syncytial system such as muscle, is currently under study in our laboratory.

The myofibers of the denervated muscle of rats treated with exogenous hormone (Group B), however, were able to maintain their length at control levels (12.31 ± 0.92 mm vs. 10.99 ± 0.54 mm). The explanation for this finding is not obvious but may well be related to the steady, aperiodic delivery of hormone to the muscle.

Testosterone replacement resulted in a myofiber nuclear content approximately four times higher than in the androgen-deprived muscles. The value obtained also exceeded that of muscles from normal (innervated and gonad-intact) as well as denervated and gonad-intact rats (Nnodim, 1999). This level of nuclear enrichment in the

TABLE 3. Effect of androgen replacement on the satellite cell population of denervated levator ani muscle in castrated rats

Animal group	Nuclear count (myofiber^{-1}) [X]	Satellite cell ratio (%) [Y][a]	Whole muscle data		
			Myofiber population[b] [Z]	Total nuclei (X.Z) × 10^6	Satellite cell population (X.Y.Z) × 10^3
Normal (gonad- and nerve-intact)	861.7	1.91 ± 0.62		5.31	101.4
A (denervated, castrated)	271.0	2.28 ± 0.23		1.67	38.08
B (denervated, castrated, treated)	1,285.8	5.15 ± 0.83	6,165 ± 11.5	7.93	408.4

[a]Statistically significant differences ($P < 0.05$), B vs. A, B vs. Normal. A vs. Normal is statistically non-significant: $P > 0.05$.
[b]Stability of myofiber numbers across experimental groups is assumed on the basis of reports by Venable (1966) and Viguie et al., (1997). Value given as mean ± standard error of three muscles.
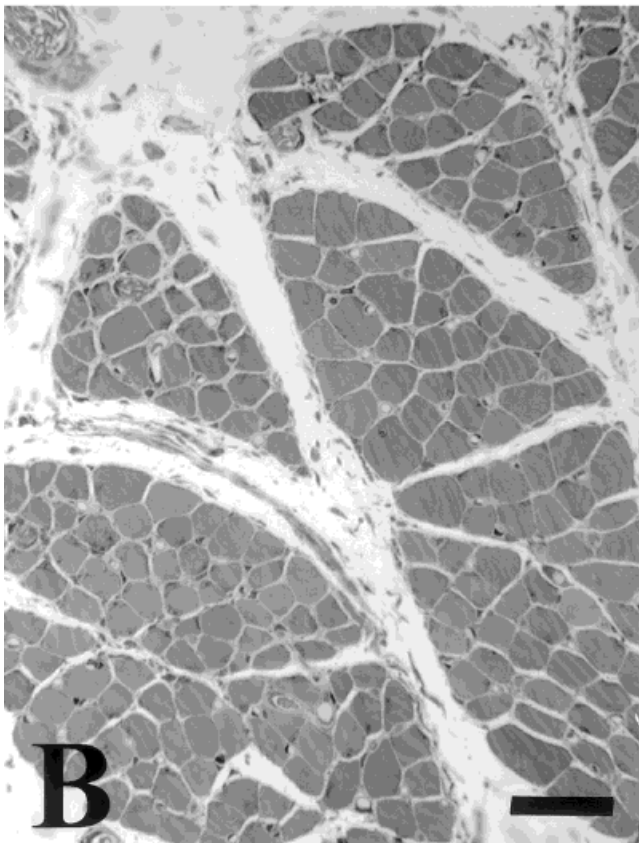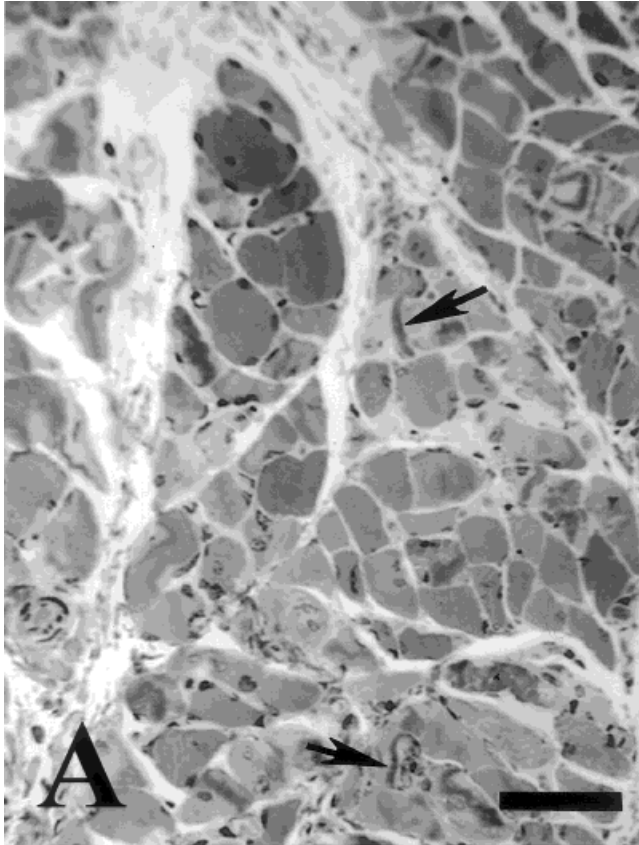
LA of Group B rats can be ascribed to the expansion of their satellite cell pool (Table 3). Autoradiographic studies by Moss and Leblond (1971) showed mitotic activity within this pool to be the mechanism of postnatal increase in myonuclei.

The above changes indicate the very wide morphological range across which the LA responds to manipulations of its trophic inputs. These changes are mirrored in studies that have considered alterations in whole-muscle wet weight (Venable, 1966; Gori et al., 1967; Bass et al., 1969). Gori et al. (1967) found that after castration, muscle weight was nearly halved (55% of normal) in 15 days, decreasing to 39.5% in 1 month and 15.6% in 8 months. At the ultrastructural level, the overall integrity of the contractile apparatus was qualitatively preserved. Equally dramatic are the results in the opposite direction. In ten-month-old rats neutered before puberty, LA wet weight and myofiber cross-sectional area more than doubled in 15 days after an injection of 1.0 mg of depot testosterone and no alteration of the structural pattern in the myofibers was observed (Venable, 1966). Other than its more rapid pace of evolution, denervation atrophy, according to Gori et al. (1967), was not substantially different from castration atrophy. Nuclear population dynamics, however, are dissimilar in both models (Nnodim, 1999), with androgens playing a significant permissive role.

Joubert et al. (1994) have studied the effects of exogenous testosterone on the LA of prepubertal rats. An increase in the satellite cell population was noted but after the age of 51 days, these cells were reported to be no longer responsive to testosterone. The underlying reason is not known. The quiescent satellite cells in mature LA remain able to respond mitotically, like other skeletal muscles, however, to such an event as denervation (Nnodim, 1999), provided circulating androgen is present. The present study confirms this enabling role of androgen.

Our knowledge of the mechanism whereby the loss of neural input induces satellite cells to reenter the cell cycle remains speculative. According to one formulation (Bischoff, 1998), proximity to electrically active sarcolemma prevents satellite cells from responding to mitogens. With denervation, this suppression is presumably lifted and satellite cells become able to enter and progress through the cell cycle under the influence of growth factors, notably fibroblast growth factor (Allen and Boxhorn, 1989; Yablonka-Reuveni et al., 1999) and hepatocyte growth factor/scatter factor (Tatsumi et al., 1998; Miller et al., 2000; Sheehan et al., 2000).

The site of action of testosterone, in the context of satellite cell dynamics, also remains undetermined. Theoretically, the hormone may either act directly on the satellite cells themselves or indirectly at a proximal target such as the associated myofiber or its motoneuron. The presence of androgen receptors in LA myofibers is well-documented (Jung and Baulieu, 1972; Krieg et al., 1974). In electrophysiological experiments using LA myofibers (Vyskocil

Fig. 1. Digitized light microscopic images of cross-sections of myofibers in the denervated levator ani muscle of rats after 2 months of castration with (**B**) and without (**A**) testosterone replacement. Note the greater degree of myofibrillar disorganization (↑) in the myofibers of the hormone-deficient group (A). Staining: Toluidine blue. Scale bar = 75 μm.

and Gutmann, 1977), input resistance was the only property found to be significantly affected by castration. A twofold increase was noted and this change was reversed when muscle weight was restored by treatment with exogenous testosterone. The relevance of this finding, if any, to the responsiveness of satellite cells is unexplored.

The LA is innervated by the axons of motoneurons in the so-called spinal nucleus of the bulbocavernosus (SNB; Breedlove and Arnold, 1980). These motoneurons are able to accumulate androgens during the perinatal period, a property that enhances their survival in the male and thus ensuring the sexual dimorphism of the perineal striated muscle complex of which the LA is a component (Nordeen et al., 1985; Jordan et al., 1991). There is no report, however, that the SNB retains its androgen sensitivity beyond the first four postnatal weeks.

Androgen receptors have not been demonstrated in satellite cells. The brevity of the interval between testosterone treatment and the proliferative response of these cells in prepubertal rats, however, was interpreted by Joubert et al. (1994) as evidence that satellite cells possess androgen receptors.

There are no reports of androgen-growth factor interactions in the context of satellite cell activation in the LA. Evidence for such interaction exists in other androgensensitive systems with regard to receptors for epidermal growth factor (prostate; Mulder et al., 1989) and transforming growth factor (bone; Kasperk et al., 1990). More recently, the expression of hepatocyte growth factor/scatter factor (HGF/SF) has been demonstrated in osteoblasts (Blanquaert et al., 1999), stromal cells of mouse prostate (Sasaki and Enami, 1999) and myoid cells of rat testis (Catizoni et al., 1999). No studies have examined the effect of androgens on HGF/SF in these cell types as yet.

As the only source of myonuclei and new myofibers in postnatal muscle, satellite cells are highly significant in the context of atrophy reversal and recovery from injury. The elucidation of the influences that impinge on them and their responses is crucial to our overall understanding of the biology of regeneration and repair in skeletal muscle. The role of denervation as a mitogenic stimulus is well-documented (Aloisi et al., 1973; Schultz, 1978; Snow, 1983; McGeachie, 1989; Lu et al., 1997; Nnodim, 1999). The present study has shown that testosterone is instrumental to the evocation of the proliferative response in rat LA. Ongoing investigations are seeking to elaborate on the role of testosterone viz-à-viz growth factors in satellite cell dynamics in the levator ani model.

ACKNOWLEDGMENTS

LITERATURE CITED

Allen RE, Boxhorn LK. 1989. Regulation of skeletal muscle satellite cell proliferation and differentiation by transforming growth factor-beta, insulin-like growth factor I, and fibroblast growth factor. J Cell Physiol 138:311–315.

Aloisi M, Mussini I, Schiaffino S. 1973. Activation of muscle nuclei in denervation and hypertrophy. In: Kakulas BA, editor. Basic research in myology. Amsterdam: Excerpta Medica. p 338–342.

Bass A, Gutmann E, Hanzliková V, Hájek I, Syrovy I. 1969. The effect of castration and denervation upon the contraction properties and metabolism of the levator ani muscle of the rat. Physiol Bohemoslov 18:177–194.

Bischoff R. 1998. Proliferation and migration of muscle stem cells. In: Allen R, Anderson J, co-chairs. Postnatal myogenesis: satellite cells in action! Boston: Muscle Satellite Cell Workshop. p 1.

Blanquaert F, Pereira RC, Canalis E. 1999. Cortisol inhibits hepatocyte growth factor/scatter factor expression and induces c-met transcripts in osteoblasts. Am J Physiol 278:E509–E515.

Breedlove SM, Arnold AP. 1980. Hormone accumulation in a sexually dimorphic motor nucleus of the rat spinal cord. Science 210:564–566.

Carlson BM. 1973. The regeneration of skeletal muscle. A review. Am J Anat 137:119–149.

Catizone A, Ricci G, Arista V, Innocenzi A, Galdieri M. 1999. Hepatocyte growth factor and c-met are expressed in rat pubertal testis. Endocrinology 140:3106–3113.

Fischer E, Ramsey VW. 1946. Changes in protein content and in some physico-chemical properties of the protein during muscular atrophies of various types. Am J Physiol 145:571–582.

Gori Z, Pellegrino C, Pollera M. 1967. The castration atrophy of the dorsal bulbocavernosus muscle of rat: an electron microscopic study. Exp Mol Pathol 6:172–198.

Gori Z, Pellegrino C, Pollera M. 1969. The hypertrophy of levator ani muscle of rat induced by testosterone: an electron microscopic study. Exp Mol Pathol 10:199–218.

Jordan CL, Breedlove SM, Arnold AP. 1991. Ontogeny of steroid accumulation in spinal lumbar motoneurons of the rat: implications for androgen's site of action during synapse elimination. J Comp Neurol 313:441–448.

Joubert Y, Tobin C, Lebart MC. 1994. Testosterone-induced masculinization of the rat levator ani muscle during puberty. Dev Biol 162:104–110.

Jung I, Baulieu E-E. 1972. Testosterone cytosol "receptor" in the rat levator ani muscle. Nat New Biol 237:24–26.

Krieg M, Szalay R, Voigt KD. 1974. Binding and metabolism of testosterone and 5∝-dihydrotestosterone in bulbocavernosus/levator ani (BCLA) of male rats: in vivo and in vitro studies. J Steroid Biochem 5:453–459.

Kasperk C, Fitzsimmons R, Strong D, Mohan S, Jennings J, Wergedal J, Baylink D. 1990. Studies of the mechanism by which androgens enhance mitogenesis and differentiation in bone cells. J Clin Endocrinol Metab 71:1322–1329.

Lu D-X, Huang S-K, Carlson BM. 1997. Electron microscopic study of long-term denervated rat skeletal muscle. Anat Rec 248:355–365.

Mauro A. 1961. Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–498.

McGeachie JK. 1989. Sustained cell proliferation in denervated skeletal muscle of mice. Cell Tissue Res 257:455–457.

Miller KJ, Thaloor D, Matteson S, Pavlath GK. 2000. Hepatocyte growth factor affects satellite cell activation and differentiation in regenerating skeletal muscle. Am J Physiol 278:C174–C181.

Moss FP, Leblond CP. 1971. Satellite cells as a source of nuclei of growing rats. Anat Rec 170:421–436.

Mulder E, Van Loon D, De Boer W, Schuurmans ALG, Bolt J, Voorhorst MM, Kuiper GGJM, Brinkmann AO. 1989. Mechanism of androgen action: recent observations on the domain structure of androgen receptors and the induction of EGF-receptors by androgens in prostate tumor cells. J Steroid Biochem 32:151–156.

Nnodim JO. 1999. Quantitative study of the effects of denervation and castration on the levator ani muscle of the rat. Anat Rec 255:324–333.

Nnodim JO. 2000. Satellite cell numbers in senile rat levator ani muscle. Mech Ageing Dev 112:99–111.

Nordeen EJ, Nordeen KW, Sengelaub DR, Arnold AP. 1985. Andro-
    gens prevent normally occurring cell death in a sexually dimorphic
    spinal nucleus. Science 229:671–673.

Sasaki M, Enami J. 1999. Hepatocyte growth factor supports andro-
    gen stimulation of growth of mouse ventral prostate epithelial cells
    in collagen gel matrix culture. Cell Biol Int 23:373–377.

Schultz E. 1978. Changes in the satellite cells of growing muscle
    following denervation. Anat Rec 190:299–311.

Sheehan SM, Tatsumi R, Temm-Grove CJ, Allen RE. 2000. HGF is an
    autocrine growth factor for skeletal muscle satellite cells in vitro.
    Muscle Nerve 23:239–245.

Snow MH. 1983. A quantitative ultrastructural analysis of satellite
    cells in denervated fast and slow muscle of the mouse. Anat Rec
    207:593–604.

Tatsumi R, Anderson JE, Nevoret CJ, Halevy O, Allen RE. 1998.
    HGF/SF is present in normal adult skeletal muscle and is capable of
    activating satellite cells. Dev Biol 194:114–128.

Venable JH. 1966. Constant cell populations in normal, testosterone-
    deprived and testosterone-stimulated levator ani muscles. Am J
    Anat 119:263–270.

Viguie CA, Lu D-X, Huang S-K, Rengan H, Carlson BM. 1997.
    Quantitative study of the effects of long-term denervation on the
    extensor digitorum longus muscle of the rat. Anat Rec 248:346–
    354.

Vyskocil F, Gutmann E. 1977. Electrophysiological and contractile
    properties of the levator ani muscle after castration and testos-
    terone administration. Pflügers Arch Eur J Physiol 368:105–
    109.

Wainman P, Shipounoff GC. 1941. The effect of castration and testos-
    terone propionate on the striated perineal musculature in the rat.
    Endocrinology 29:975–978.

Yablonka-Reuveni Z, Seger R, Rivera AJ. 1999. Fibroblast growth
    factor promotes recruitment of skeletal muscle satellite cells in
    young and old rats. J Histochem Cytochem 47:23–42.