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Quantitative Study of the Effects of Denervation and Castration on the Levator Ani Muscle of the Rat

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ABSTRACT

The levator ani muscle (LA) of the rat is highly androgen-sensitive and, like all skeletal muscles, deteriorates structurally and functionally when denervated. In order to elucidate the interplay of neural and endocrine influences, the separate and combined effects of denervation and castration on myofiber cross-sectional area and nuclear populations were quantitatively studied.

In one group of 4-month-old male rats (A), the LA was denervated. Another group (B) was surgically castrated and a third group (C) was both denervated and castrated. The control rats (D) remained both gonad- and nerve-intact. After two months, the LA was obtained for myofiber and nuclear enumeration, cross-sectional area and satellite cell frequency determination.

In the denervated muscle of gonad-intact rats (Group A), myofiber cross-sectional area was markedly diminished (265.84 ± 11.38μm^2; compared with controls [Group D]: 1519.98 ± 79.41μm^2; $P < 0.05$). Satellite cell nuclei, as a percentage of total sublaminar nuclei (i.e., satellite cell ratio), increased significantly (4.26%, from a control value of 1.91%). Castration alone (Group B) resulted in pronounced myofiber atrophy (mean cross-sectional area: 754.03 ± 89.63μm^2) but had no significant effect on satellite cell ratio (2.36%). The combination of castration and denervation (Group C) elicited the same degree of myofiber atrophy as denervation alone (Group A) but had no significant impact on satellite cell ratio. Instead, the nuclear count per myofiber declined to about a third of the control level (300.5 ± 38.49 compared with 861.7 ± 24.8; $P < 0.05$).

The results indicate that the atrophic effects of denervation and castration on the LA are non-synergistic and mechanistically similar. They also show that the inability of satellite cells to respond mitotically to the withdrawal of neural input under disandrogenized conditions is a factor in the myonuclear depletion of the denervated muscle of castrated rats. Anat Rec 255:324–333, 1999.

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

Skeletal muscles are drastically affected by the loss of their motor innervation. Among the changes reported in limb muscles is the re-entry of satellite cells into the cell cycle (Aloisi et al., 1973; Ontell, 1974; McGeachie and Allbrook, 1978; Snow, 1983; Rodrigues and Schmalbruch, 1995; Lu et al., 1997; Viguie et al., 1997). These cells proliferate and either differentiate into new muscle fibers or contribute myonuclei to regenerating ones.

There are no reports on satellite cell responses in denervated rat levator ani muscle (LA), but the atrophic changes described are similar to those that occur in limb muscles (Bass et al., 1969; Buresová et al., 1972). Unlike limb muscles, however, rat LA is markedly androgen-

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sensitive (Wainman and Shipounoff, 1941; Eisenberg and Gordan, 1950; Joubert and Tobin, 1989). Numerous studies on adult rats have documented a steep reduction in muscle mass as well as ultrastructural disruption post-castration (Gori et al., 1967, 1969; Bass et al., 1969; Galavazi and Szirmai, 1971). These same studies also demonstrated a rapid reversal of the castration-induced changes after the administration of exogenous testosterone, with full restoration of form and function. Studies by Joubert and Tobin (1989, 1995) and Joubert et al. (1994) suggest that endocrine status has an impact on satellite cells in the LA of prepubertal male rats as well as the vestiges of the muscle in adult females. However, there are no published data on the effect of androgen deprivation on the satellite cell population in the LA.

As a target organ of both the voluntary motor and endocrine systems, the LA is uniquely well-suited to the experimental modeling of both post-denervation and post-castration atrophy in the evaluation of the interplay of neural and hormonal influences on muscle. In the present study, satellite cell responses in denervated adult rat levator ani, under normal and androgen-deprived conditions, are reported.

MATERIALS AND METHODS

Four groups (A - D) of 4-month-old male rats of the WI/HicksCar strain were used for the present study. In groups A and C, pelvic access was gained via a midline scrotal incision, and the pudendal nerve was divided between silk ligatures (6.0; Ethicon, Somerville, NJ) on both sides. The proximal stump was reflected and buried in the body wall to prevent regrowth into the LA. Animals in groups B and C were surgically castrated by bilateral orchidectomy. The control group (D) consisted of normal rats with intact gonads and innervated LA. All procedures were carried out under ether inhalation anesthesia and the rats received oral tetracycline (dissolved in their drinking water) postoperatively for one week.

Eight weeks later, the animals were reanesthetized and the LA was dissected out and processed for myofiber counting (three animals in Group D), fluorescence microscopic analysis of dissociated muscle fibers (three animals per group) as well as light and electron microscopy (three animals per group).

Animal care and use protocols were in conformity with NIH guidelines. Food and water were provided ad libitum and a 12/12 light/dark cycle was maintained using automatic switching devices. Terminally, the animals were euthanized by an overdose of inhaled ether.

Single Fiber Isolation and Nuclear Enumeration

Individual muscle fibers were obtained by a connective tissue digestion technique developed in the Carlson Laboratory at the University of Michigan. The muscle was splinted at resting length on slotted cardboard and incubated in a solution of 0.45% collagenase type 3 (Worthington, Freehold, NJ) in phosphate-buffered saline (PBS; pH 7.4) with constant agitation for 75 min at 37°C. It was then rinsed with three changes of PBS and fixed for 10 min in 2% paraformaldehyde (pH 7.4) at room temperature, followed by another rinse cycle.

Using fine probes, entire single myofibers were obtained from the outer zone of the muscle by careful microdissection under a dissecting microscope and transferred to a 40µm-mesh cell strainer (Becton-Dickinson, Franklin Lakes, NJ) set in a petri dish containing 9.0 ml of PBS. One ml of 0.1% propidium iodide was added followed by trituration, and nuclear labeling was allowed to occur over a 10-min period in the dark. The fibers were then rinsed in three changes of PBS and mounted on glass slides in an antifading agent (Permafluor; Lipshaw Immunon, Pittsburgh, PA). Struts were interposed between the coverslip and slide to preserve their three-dimensional properties. The LA from three rats was used for this part of the investigation and five single fibers, devoid of connective tissue contamination, were prepared per muscle.

Imaging and digitization were performed in an inverted microscope (Zeiss Axiovert 135TV) coupled to a Power-Macintosh 7300/200 computer workstation (Apple Computer Inc.) fitted with a frame grabber (Scion Corp.) driven by the public-domain NIH Image program (U.S. National Institutes of Health). At a wavelength of 617 nm and 20× eyepiece magnification, successive overlapping segments of muscle fiber were imaged in multiple focal planes, creating a stack of optical slices for each segment.

After spatial calibration, the NIH Image program was used to determine nuclear numbers and fiber lengths semi-automatically. First, the boundaries of each myofiber segment were delineated on the most favorable slice in the stack and the segment length was measured. The nuclei were then marked, using the cross-hair tool. Reference was made to the other slices as necessary to ensure that no nuclei were omitted or marked more than once. The measurement counter kept track of the exercise and displayed the total number of nuclei (myonuclei plus satellite cells) on command. The data (nuclear counts and segment lengths) were finally exported to a tab-delimited text file in the Excel program (version 5.0, Microsoft Corp.) for further analysis. Nuclear counts per unit length of myofiber were computed for both the entire myofiber and its subregions (bulbar, middle, and rapheal thirds).

Myofiber Enumeration

Myofibers in the LA were counted after nitric acid digestion as described by Blaivas and Carlson (1991). The muscle was placed in 15% nitric acid for 24 hours to degrade the connective tissue. It was then transferred to a 1:1 mixture of glycerol and 2% sodium dodecyl sulfate for examination under a dissecting microscope. Using watchmaker's forceps, individual myofibers were gently loosened, and directly counted. The LA from three control rats (Group D) was used for this part of the study.

Myofiber Cross-Sectional Areas and Satellite Cell Analysis

The LA from three rats in each group were obtained for aldehyde fixation and resin embedding. After removal from the animal, the muscle was splinted at resting length on a piece of slotted cardboard and immerse-fixed in a solution of 2.5% glutaraldehyde and 4% formaldehyde in 0.1M phosphate buffer (pH 7.4) for one hour at 4°C. In the middle third of the muscle, the outer zone (the region from which single myofibers were isolated in other rats) was then diced into cylinders approximately 0.5 mm x 1.0 mm in size to facilitate orientation during embedding and sectioning. Thereafter, the samples were returned to fresh fixative and kept overnight at 4°C. Following postfixation

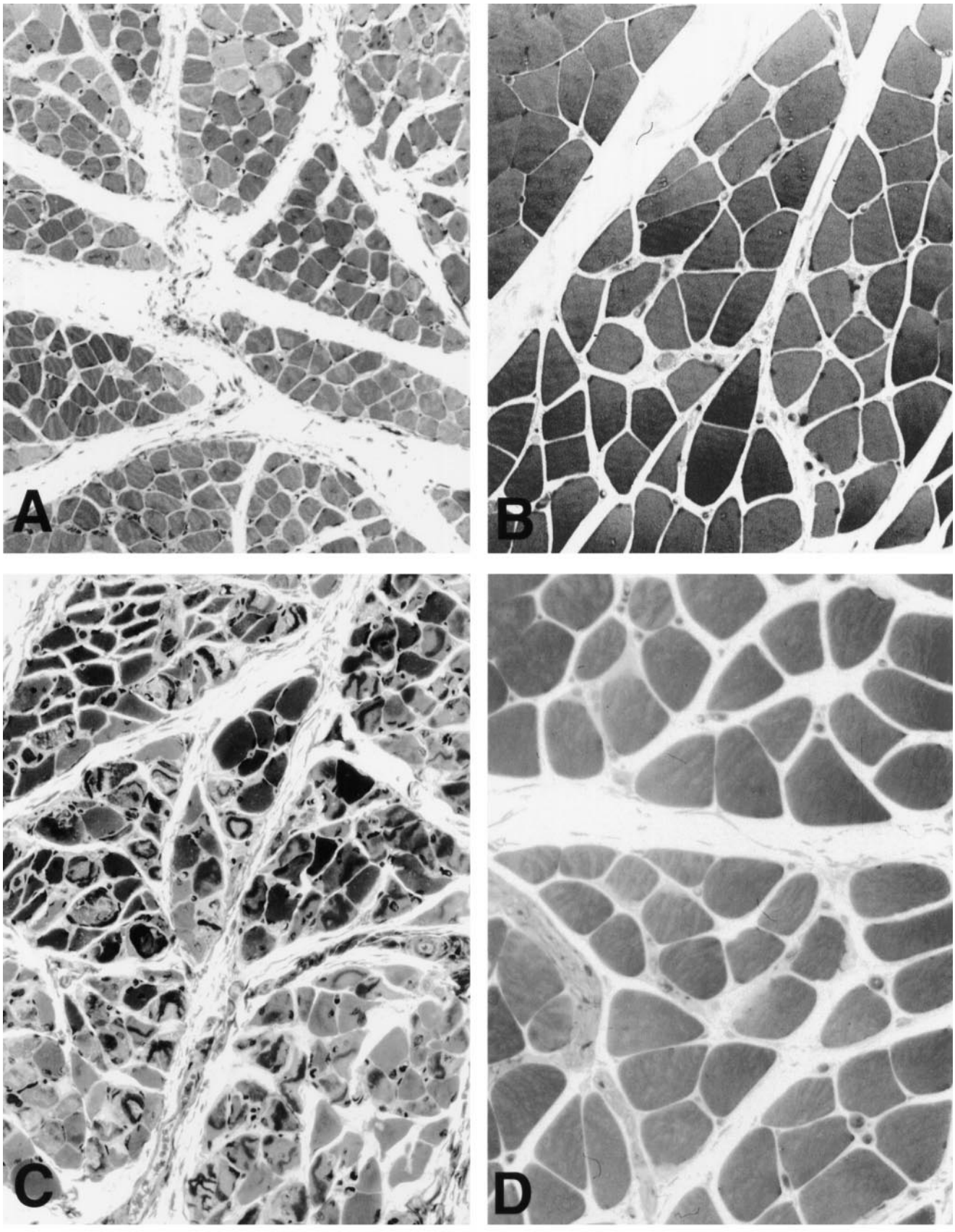

Fig. 1. Digitized light microscopic images of cross-sections of myofibers in the levator ani muscle of normal rats (**D**) and after two months of denervation (**A**), castration (**B**), and denervation-*cum*-castration (**C**). Note the advanced atrophy of myofibers in (A) and (C) compared with (D). Staining: Toluidine blue. Scale bar = 75µm.

in 1% buffered osmium tetroxide, they were dehydrated in a graded series of ethanol and embedded in Spurr resin.

Five blocks were randomly selected for each animal and both semi-thin (1.5µm-thick) and ultra-thin (70 nm-thick) sections were prepared from each block. The semi-thin sections were mounted on glass slides and stained with toluidine blue for myofiber cross-sectional area determination by computer-assisted light microscopic planimetry. The ultra-thin sections were picked up on 200-mesh copper grids and double-stained with uranyl acetate and lead citrate for examination in a Philips CM-100 digital transmission electron microscope.

The images of myofiber cross-sections in the semi-thin sections were captured using a digitizing video camera (PVC 100C; Pixera Corp.) fitted to a Leitz Diaplan light microscope and interfaced with a Apple PowerMacintosh 7300/200 workstation running the NIH Image program. After spatial calibration, the freehand drawing tool and an electronic pen pointing device (Wacom Technology Corp.) were used to outline individual myofibers and determine their cross-sectional area. For each animal group, a total of at least 350 fiber profiles were measured and their mean area computed.

One grid per block was examined by electron microscopy and a total of at least 1,000 sublaminal nuclei per animal group identified and tabulated as either satellite cell nuclei or myonuclei. The frequency of satellite cells was expressed as a percentage of the total number of sublaminal nuclei visualized.

For comparison, the extensor digitorum longus muscle (EDL) of three castrated rats was denervated by sciatic neurotomy. After eight weeks, the muscle was harvested and processed for electron microscopy as described above.

Comparisons of means were made among the different animal groups and between pairs of groups using one-way ANOVA and Student's t-test respectively, with statistical significance set at $P < 0.05$.

RESULTS

Myofiber Cross-Sectional Area

The muscle fibers from all the experimental groups (A - C) showed a marked degree of atrophy, compared with those from the control animals (D), by inference from micrographic (Fig. 1) as well as cross-sectional area data (Table 1). The most profound effect was observed in denervated muscle (Group A: 265.84µm^2 ± 11.38 vs. Group D: 1,519.98µm^2 ± 79.41). Castration alone had a considerable but much lesser impact than denervation (Group B: 754.03µm^2 ± 89.63). The mean cross-sectional area of myofibers in the denervated LA of castrated rats (Group C: 311.75µm^2 ± 48.62) was not significantly different from that of denervated muscle fibers of gonad-intact rats (Group A; $P > 0.05$).

Figure 2 depicts the frequency distribution of myofiber cross-sectional areas in the different animal groups, the modal values closely reflecting the left-shift with denervation and/or castration as well as the similarity in the effect of denervation on the one hand and denervation-*cum*-castration on the other (200 - <300µm^2). In control rats (Group D), the majority of fibers were in the 1,200 - <1,300µm^2 size range. After castration alone (Group B), very few fibers in excess of 1,200µm^2 in cross-sectional area were seen. In the denervated muscle of gonad-intact (Group A) or castrated rats (Group C), there were no fibers larger than 1,200µm^2.

TABLE 1. Myofiber cross-sectional areas

Animal group	Area (µm^2)
A	265.84 ± 11.38
B	754.03 ± 89.63
C	311.75 ± 48.62
D	1,519.98 ± 79.41

Values given as mean ± standard error.
Statistically significant differences ($P < 0.05$): D vs. A, B, C; B vs. A, C.
Statistically non-significant difference: A vs. C.

Nuclear Count

Denervation (Group A: 151.33 mm^{-1} ± 7.5) and castration (Group B: 120.81 mm^{-1} ± 5.8) resulted in an increase in the linear nuclear density of myofibers in comparison with controls (Group D: 79.58 mm^{-1} ± 3.5; $P < 0.05$). However, the combination of denervation and castration (Group C: 82.38 mm^{-1} ± 8.8) had no statistically significant effect (Table 2).

The myofibers exhibited restricted areas of high nuclear density, especially in the experimental groups (A - C). In the control group (D), localized nuclear aggregations were observed at the bulbar extremity and the neuromuscular junction (Fig. 3). When thirds (rapheal, middle, and bulbar) of the fiber were considered, the regional distribution of myonuclei along the fiber was found to be relatively uniform within all groups (Table 3).

Table 4 shows the sarcoplasm-to-myonucleus ratio of myofibers computed from the average values of myofiber length, cross-sectional area, and nuclear content. The massive collapse in sarcoplasmic volume caused by denervation alone (Group A) resulted in a 90.9% decline in sarcoplasm-to-nucleus ratio (1.77 x 10^{3}µm^3 .nucleus^{-1}; compared with 19.38 x 10^{3}µm^3 .nucleus^{-1} for normal myofibers). The combination of denervation and castration (Group C) also had a very considerable but lesser effect (81.1% reduction to 3.66 x 10^{3}µm^3 .nucleus^{-1}). Castration alone had the least impact (66.5% decline to 6.50 x 10^{3}µm^3 .nucleus^{-1}).

Satellite Cell Count

After two months of denervation, the satellite cell population in the LA of group A rats approximately doubled (4.26% vs. Group D: 1.91%) as a percentage of the total number of sublaminal nuclei in the muscle (Table 5). In castrated rats, the satellite cell ratio remained unchanged from controls, in both denervated (Group C: 2.33%) and nerve-intact (Group B: 2.36%) muscles. This is in sharp contrast to the EDL muscle in which castration did not appear to interfere with the denervation-induced enhancement in satellite cell frequency. A satellite cell ratio of 8.33 ± 1.2% was obtained for the denervated EDL muscle of castrated rats.

Table 5 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). In the denervated muscle of gonad-intact rats, the satellite cells doubled in number (205.8 x 10^3, compared with 101.4 x 10^3 in normal LA). However, they were markedly depleted in the denervated muscle of castrated rats (43.1 x 10^3). Castration alone had no statistically significant effect on the satellite cell population (117.1 x 10^3).

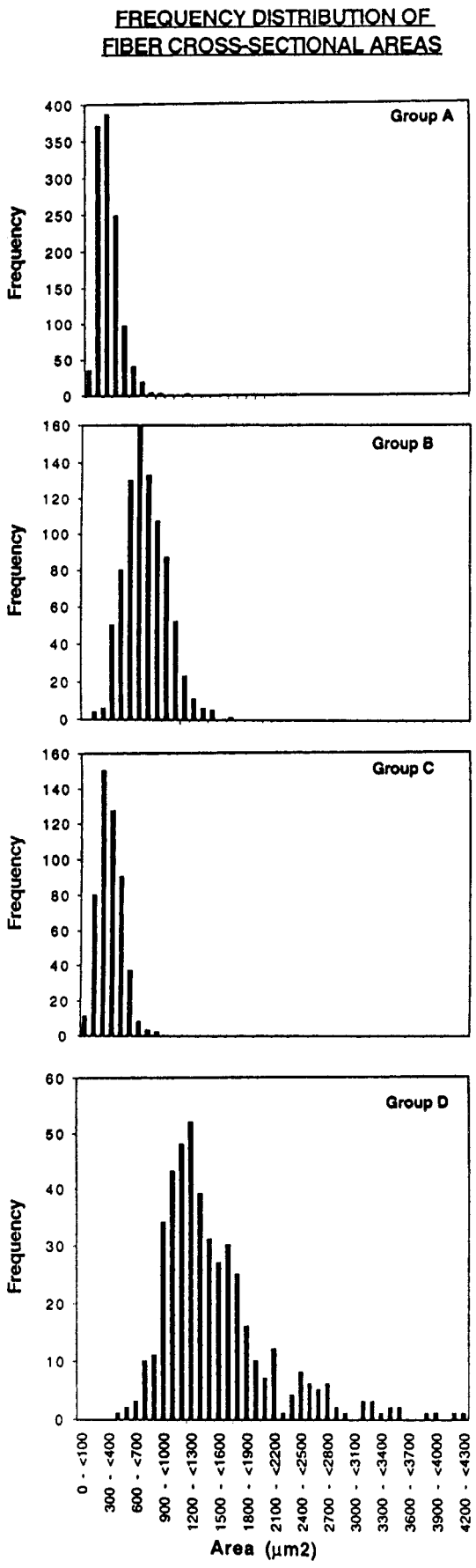

TABLE 2. Myofiber nuclear density

Animal group	Fiber length (mm)	Nuclear count	Density (mm^{-1})
A	5.23 ± 0.30	784.2 ± 45.4	151.33 ± 7.5
B	6.76 ± 0.32	804.1 ± 31.9	120.81 ± 5.8
C	3.53 ± 0.16	300.5 ± 38.4	82.38 ± 8.8
D	10.99 ± 0.54	861.7 ± 24.8	79.58 ± 3.5

Myonuclei + Satellite cell nuclei per millimeter of myofiber.
Values given as mean ± standard error.
Fifteen myofibers analyzed per group (n = 3).
Statistically significant differences ($P < 0.05$): A vs. C, D; B vs. D.

DISCUSSION

In skeletal muscles generally, the nerve supply is of crucial importance to structural and functional integrity (Gutmann and Zelená, 1962; Gulati, 1990; Lewis and Schmalbruch, 1994). However, the LA of rats is one of very few mammalian skeletal muscles known to be highly and specifically sensitive to androgens (Wainman and Shipounoff, 1941; Jung and Baulieu, 1972) in addition. Quite conceivably, both influences (neural and endocrine) might interact but this possibility has hitherto not been investigated. In the present evaluation, the approach adopted was to withdraw one or both agencies and then quantify the resultant effects based on changes in three parameters: myofiber cross-sectional area, myonuclear content, and satellite cell population.

The myofiber cross-sectional area in denervated LA decreased to 17.5% of control values in eight weeks (Table 1). Comparable results were obtained by Bass et al. (1969) who measured atrophy in terms of relative wet weight loss but using younger animals. In rats denervated at the age of one month, they noted that the relative wet weight of the LA had fallen to 10% of control levels after two months. With castration however, the decline was a third less (i.e., to 30% of normal), also proportionately similar to the results obtained in the present study and consistent with the observation by Souccar et al. (1982) that the effects elicited were inversely related to the age of the animal.

The present data also show that the sarcoplasmic responses of LA to denervation and castration are not additive. Whereas both insults, applied concurrently, induced a more profound degree of atrophy than castration alone, their combined effect was no more than that of denervation alone. It would seem that denervation interferes with the same set of cellular processes that are susceptible to castration as well as others that are hormone-insensitive. An indication of such qualitative dissimilarity is the observation by Bass et al. (1969) that denervation and castration could each depress the myofibrillar protein content of LA globally but only denervation altered the sarcoplasmic-to-contractile protein and oxidative-to-glycolytic enzyme ratios. However, with regard to satellite cells, the nature of the interaction appears to be different. As discussed below, castration had no direct effect on satellite

Fig. 2. Histogram showing the frequency distributions of myofiber cross-sectional areas in rat LA muscle. Atrophy is indicated by the displacement of the mode from 1,200 - <1,300µm^2 in the control group (**D**), to size classes within the 200 - <700µm^2 range in the experimental groups. (**A**–**C**). Denervation with (C) or without (A) castration had the most profound effect.

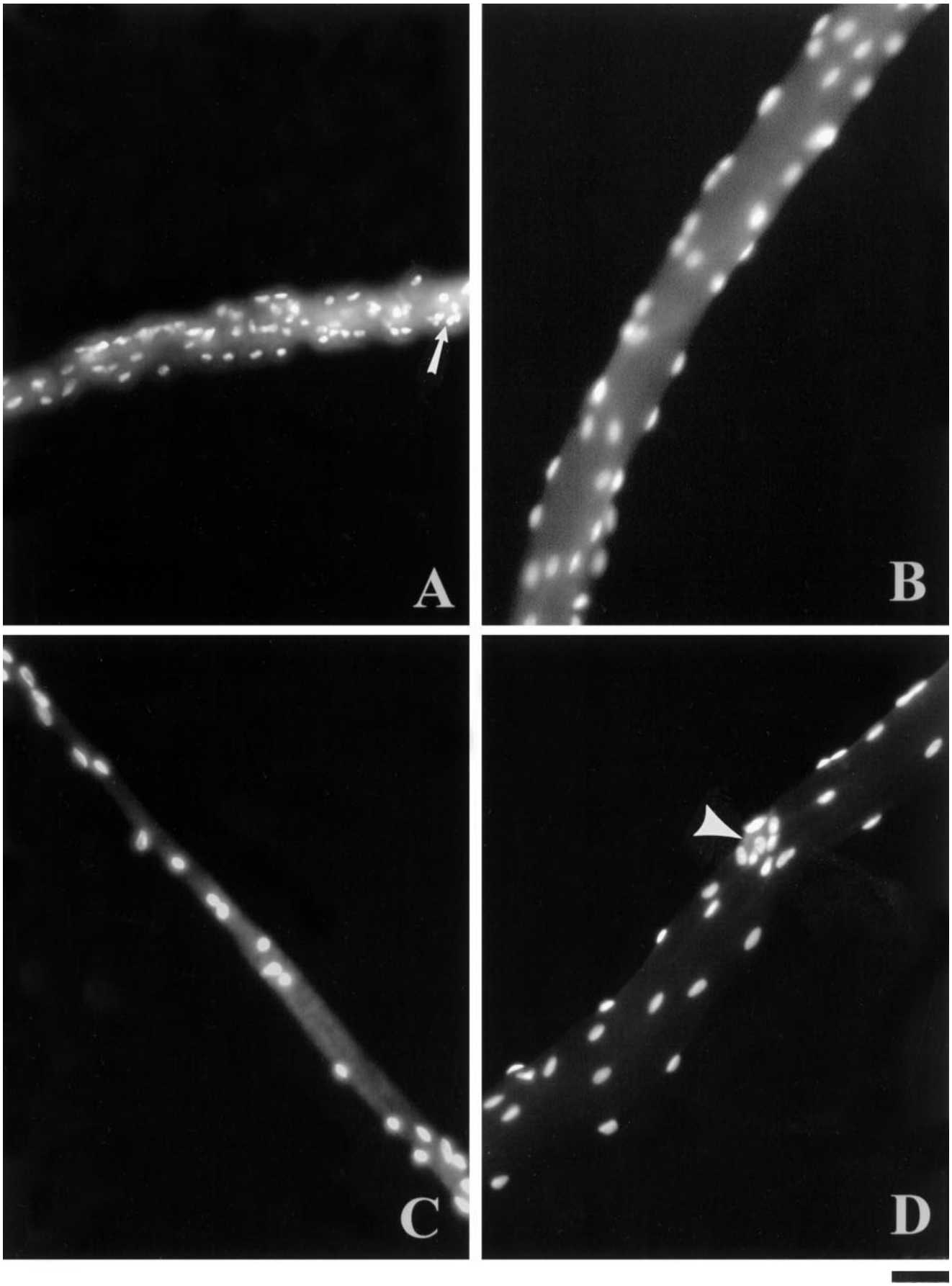

Figure 3. (legend, overleaf.)

TABLE 3. Nuclear density by myofiber region

Animal group	Nuclear density (mm^{-1})		
	Bulbar third	Middle third	Rapheal third
A	154.38 ± 12.3	148.43 ± 6.2	251.86 ± 95.6
B	125.91 ± 8.4	136.16 ± 6.0	103.07 ± 0.4
C	89.95 ± 6.0	81.96 ± 12.2	76.57 ± 11.2
D	84.92 ± 5.2	81.44 ± 5.7	79.23 ± 3.3

Myonuclei + Satellite cell nuclei per millimeter of myofiber.
Values given as mean ± standard error.
Intragroup disparities are statistically negligible.

cells, but was able to hinder the mitogenic impact of denervation.

Myofibers are very long cells and are in relationship with a variety of non-muscle elements along their length. At their extremities for instance, they interface with tendon fibroblasts and, in between, with axon terminals, Schwann cells, capillary pericytes and other cell types. These relationships are known to impose regionally unique synthetic demands on the myofiber, a classic illustration of which is the abundance of acetylcholinesterase receptor mRNA in the subsynaptic cytoplasm, compared with extrajunctional parts (Merlie and Sanes, 1985). Linear nuclear densities were estimated in the present study to determine whether the distribution of nuclei along the myofiber revealed a pattern reflective of the spatial heterogeneity in synthetic requirements under normal and experimental circumstances.

In myofibers from all groups, normal and experimental, no significant differences were noted in the nuclear densities of the extreme and middle thirds (Table 3). Comparable results were obtained for rat soleus and plantaris muscles by Tseng et al. (1994).

Although the overall distribution was uniform, internuclear distances were variable. In the middle third of normal myofibers, a distinctive tight ringlet of 6 - 12 myonuclei was regularly seen at the neuromuscular junction. Clumps of myonuclei, separated by myonucleus-depleted stretches of myofiber several tens of microns long, were also frequently observed in denervated myofibers (Fig. 3). Similar appearances have been reported in long-term denervated rat extensor digitorum longus (EDL) muscle by Viguie et al. (1997).

Tseng et al. (1994) have commented on the correlation between myonuclear density and physio-biochemical fiber type. In a study of limb muscles in female Sprague-Dawley rats, myonuclear density was found to be inversely related to speed of contraction (in the "slow/fast" context) but directly proportional to oxidative metabolic capacity. Thus, slow, highly oxidative fibers had a myonuclear density over two and one half times that of fast, anerobic fibers (soleus: 116 ± 51 mm^{-1} vs. plantaris: 44 ± 23 mm^{-1}). Although strain, sex, and functional differences somewhat constrain direct comparisons, the nuclear density recorded for normal LA in the present study (79.58 ± 3.5 mm^{-1}) is intermediate between the above extremes (Table 2). This is consistent with the relatively glycolytic metabolic profile of the muscle (Bass et al., 1969) as well as its fast contraction kinetics (Arvill and Ahrén, 1966; Hanzliková and Gutmann, 1972; Vyskocil and Gutmann, 1977) and myosin isoform content (d'Albis et al., 1989, 1991; Nnodim, 1999 (submitted).

Denervation caused a drastic reduction of the sarcoplasmic volume associated with individual myonuclei (the "nuclear domain"; Pavlath et al., 1989) but that effect was somewhat mitigated by androgen deprivation in the aneural myofibers of castrated rats (Group A: 9.1% of normal vs. Group C: 18.9% of normal; Table 4). The more profound effect in denervated rats (Group A) was due to a relatively stable myonuclear content whereas massive nuclear depletion occurred in the denervated myofibers of castrated rats (Group C). In both groups, the degrees of atrophy suffered by the myofibers, as inferred from their cross-sectional areas, were statistically similar. As discussed below, the reason denervated myofibers were able to maintain their myonuclear population, in all probability, resides in the response of their satellite cells. The least deficit in sarcoplasm-to-nucleus ratio was noted in disandrogenized (but nerve-intact) myofibers (Group B: 33.5% of normal). No significant change in the myonuclear population occurred in these fibers and the collapse in sarcoplasmic volume was less drastic than in the denervated groups (A and C).

It is unclear what the adaptive significance, if any, of a reduction in nuclear domain size might be in the altered circumstance of denervated and castrated myofibers. In rat hindlimb muscles, Kasper and Xun (1996) also noted a marked decline in sarcoplasm-to-nucleus ratio, especially in fast myofibers, after four weeks of limb suspension. They speculated that the reduction might meet the need to shrink diffusion distances for mRNA or down-regulate transcriptional control under conditions of diminished functional demand.

In the present study, denervation resulted in a two- to threefold increase in satellite cell ratio (from 1.91% [Group D] to 4.26% [Group A]; Table 5). Viguie et al. (1997) observed that myofibers in rat EDL remained numerically stable through seven months of denervation. To the extent that it is valid to assume the same for the LA, the total satellite cell population in the muscle was found by calculation to have doubled after two months of denervation (Table 5). Satellite cell multiplication and differentiation into new myofibers or incorporation into regenerating ones might account for the maintenance of stable myofiber and myonuclear numbers. The proliferation of satellite cells in response to denervation has been extensively documented in limb muscles (Aloisi et al., 1973; Ontell, 1975; McGeachie and Allbrook, 1978; Schultz, 1978; Murray and Robbins, 1982; Snow, 1983; McGeachie 1989; Rodrigues and Schmalbruch, 1995; Lu et al., 1997; Viguie et al., 1997).

Although castration alone had no effect on satellite cell frequency, androgen deprivation inhibited satellite cell multiplication in denervated LA (Table 5). The mechanism of this intriguing finding is unknown. It must be viewed, however, against a background of the principles underlying satellite cell quiescence in nerve-intact muscle. Ongoing investigations in this field are evaluating electrical activity and humoral factors.

Fig. 3. Fluorescence micrographs of representative segments of isolated LA myofibers. The atrophic condition induced by denervation (**A**) and denervation-*cum*-castration (**C**) is evident, compared with the normal (**D**). Localized aggregations of nuclei (⇑) are commonly seen but overall distribution appears to be uniformly random. (⇑): subsynaptic myonuclear cluster at a neuromuscular junction. Nuclear labeling: Propidium iodide. Scale bar = 50μm.

TABLE 4. Effect of denervation and castration on sarcoplasm-to-myonucleus ratio

Animal group	Nuclear count (myofiber^{-1})	Myofiber length ($\times 10^3$ μm)[1]	Myofiber cross-sectional area (μm^2)[1]	Myofiber (sarcoplasmic) volume ($\times 10^6$ μm^3)	Sarcoplasm-to-myonucleus ratio ($\times 10^3$ μm^3 · myonucleus^{-1})
A	784.2	5.23	265.84	1.39	1.77
B	804.1	6.67	754.03	5.10	6.50
C	300.5	3.53	311.75	1.10	3.66
D	861.7	10.99	1,519.98	16.70	19.38

Means from Tables 1 and 2.
Although Groups A and C lost sarcoplasmic volume approximately to the same extent, the myonuclear population remained relatively stable in Group A but was depleted in Group C.

TABLE 5. Effects of denervation and castration on the satellite cell population

			Whole muscle data		
Animal group	Nuclear count (myofiber^{-1}) [X]	Satellite cell ratio (%) [Y]	Myofiber population [Z]	Total nuclei (X.Z.) $\times 10^6$	Satellite cell population (X.Y.Z) $\times 10^3$
A	784.2	4.26		4.83	205.8
B	804.1	2.36		4.96	117.1
C	300.5	2.33		1.85	43.1
D	861.7	1.91	6165 ± 11.5	5.31	101.4

Means from Table 2.
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.
The results show the sharply contrasting effects of denervation alone (augmentation; Group A) and denervation with castration (depletion; Group C) on absolute satellite cell numbers.

Füchtbauer and Westphal (1992) noted that the myogenic determination factors MyoD1 and myogenin, which were immunohistochemically undetectable in normal mouse tibialis anterior muscle, appeared in mononucleated cells within newly formed myotubes during muscle regeneration. MyoD1 and myogenin mRNA have been detected also in mononucleated cells within crushed (Grounds et al., 1991) and denervated (Eftimie et al., 1991) muscle within hours of injury and, in experiments by Eftimie and colleagues (1991), the augmentation in the levels of these transcripts in denervated rat soleus muscle was repressed by direct electrical stimulation using external electrodes.

The mononucleated cells referred to in the above studies were presumed to be satellite cells. However, given the heterogeneity of the cellular composition of skeletal muscle, a precise identification is not possible other than by electron microscopy. Further, it is unclear whether the electrical signals act directly on satellite cells or through the intermediary of a primary effect on the associated myofiber.

It would be interesting to attempt a replication of these limb muscle observations in the LA so as to be able to introduce androgen deprivation into the picture. However, according to Vyskocil and Gutmann (1977), the only electrophysiological deficit induced by castration is a somewhat undramatic increase in input resistance (0.31 ± 0.02MΩ to 0.73 ± 0.04MΩ) over a six-month period. This alteration was attributed to the reduction in diameter of the atrophic myofibers and disorganization of their sarcotubular system. The values were restored to normal with recovery of muscle weight following treatment with testosterone.

Other speculations on the rekindling of satellite cell activity by denervation have focused on growth factors. According to McGeachie (1989) and Bischoff (1990), these factors are able to release satellite cells from the inhibitions imposed on them by myofiber activity. On the basis of in vitro studies by Allen and Boxhorn (1989) and Greene and Allen (1991), transforming growth factor-beta (TGF-B), beta-fibroblast growth factor (B-FGF) and insulin-like growth factor-1 (IGF-1) have emerged as leading contenders for key regulatory roles in satellite cell dynamics. Transforming growth factor has been shown to inhibit satellite cell proliferation and differentiation. It is opposed by B-FGF, which, however is ordinarily bound to proteoglycans in the basal lamina. Denervation is presumed to somehow cause the activation of proteolytic enzymes which then liberate B-FGF from the basal lamina to override TGF-B. Insulin-like growth factor-1 sustains the proliferative process in the role of a progression factor.

More recently, hepatocyte growth factor/scatter factor (HGF/SF), a 90kDa disulfide-linked heterodimer of as-yet-uncertain derivation, has been identified in the extracellular matrix of normal adult rat limb muscles and implicated in a modulatory role with regard to satellite cell activity (Tatsumi et al., 1998). However, more evidence is needed before a physiologic significance can be ascribed to some or all of the regulatory peptides currently under consideration. Whatever the case may be though, the results of the present study indicate that in the specific case of the LA, testosterone is a crucial adjunct to denervation for the realization of a mitogenic effect in vivo.

A role for androgens in satellite cell activation in denervated limb muscles is not substantiated by the results of the present study. A satellite cell frequency of 8.33 ± 1.2% was recorded for denervated EDL muscle in castrated rats. This is comparable to 9.1 ± 0.1% obtained by Viguie et al. (1997) for the same muscle when denervated in gonad-

intact rats. Depending on the parameter chosen, similarities and dissimilarities between satellite cells from different sources have been documented. Thus, Dusterhoft et al. (1990) noted different patterns of myosin light chain expression in colonies of satellite cells from rat diaphragm, soleus, and tibialis anterior muscles, but identical characteristics with regard to myosin heavy chain synthesis. Hughes and Blau (1992) showed that satellite cells fuse indiscriminately with myofibers and, according to Schultz and McCormick (1994), environmental factors in vivo tend to prevail over any intrinsic differences among satellite cells, rendering them homogeneous. Clearly, satellite cells from the LA need to be studied both in vivo and in vitro in order to fully elucidate their biological characteristics.

Interest in satellite cell biology is currently driven by the potential for the use of these cells as a source of myoblasts for implant therapy regimens in such skeletal muscle diseases as Duchenne dystrophy. Crucial to the actualization of this potential is a better understanding of the numerous factors that influence satellite cell activity in relation to the extent to which their highly promising in vitro behavior can be re-enacted in vivo. It is in the above context that the inhibition of the denervation-induced satellite cell proliferation by androgen deprivation merits further consideration.

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