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Histological evidences of reparative and regenerative effects of ß-adrenoceptor agonists, clenbuterol and isoproterenol, in denervated rat skeletal muscle

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The aim of this study was to determine the contribution of ß-adrenoceptor activation in the reconstruction of the structural and functional organization of denervated skeletal muscle. ß-agonists, clenbuterol (1.2 mg/kg body weight) and isoproterenol (2 mg/kg body weight), administration (daily oral administration; maximum 7 days) to normal innervated rats as well as denervated animals caused muscle hypertrophy. An increase in mean fiber diameter confirmed this stimulated growth both in normal innervated and denervated rat gastrocnemius muscle. Examination of muscle nuclei from treated but normal innervated rat gastrocnemius exhibited features like large size, active nucleoplasm and an increase in their number per fiber cross section and per mm mean fiber length indicating towards an elevated biosynthetic activity in tissue in the presence of ß adrenoceptor agonists. Administration of drugs to normal innervated animals resulted in an emergence of central muscle nuclei. The hyperactive and enlarged muscle nuclei ultimately organized themselves into unusually elongated nuclear streaks. ß agonist treatment to denervated rats resulted in amelioration of atrophic state of tissue characterized by hypertrophy of muscle fibers thus lending to a restoration of structural organization of tissue. Bizarre shapes of nuclei in denervated muscle tend to recover to that characteristic to normal innervated muscle in presence of clenbuterol and isoproterenol hydrochloride. All observations were confirmed by administering butoxamine, a ß-adrenoceptor antagonist along with ß-agonists. The results suggests that both clenbuterol and isoproterenol hydrochloride are capable of mimicking normal innervation functions in skeletal muscle and thus play important role in the structural and functional reorganization of tissue. Amelioration of denervation atrophy in rat gastrocnemius in the presence of ß-agonists supports this.

Keywords: Butoxamine, Clenbuterol, Denervation, Isoproterenol hydrochloride, Nuclei, Rat gastrocnemius

ß-adrenergic agonists, clenbuterol, salbutamol and albuterol, are sympathomimetic substances capable of mimicking normal innervation functions in skeletal muscle. These agonists are endowed with a remarkable ability to alter structural and functional characteristics of skeletal muscle and thereby induce changes in phenotypic expression of tissue[1-5]. ß agonist induced changes in phenotypic expression of skeletal muscles suggest that the drugs have a lot of clinical potential in limiting or reversing muscle wasting conditions and other related disorders[6]. Although banned for clinical use, sports persons invariably use these drugs to enhance their performances in power related games[7,8]. Chronic administration of drugs like clenbuterol, ractopamine and isoproterenol produce changes in skeletal muscle mass[9], contractile properties[10], in addition to altered fiber dimensions, protein isoforms[4], stepped up force production with a concomitant change in shortening velocity[11]. Consequently, some ß-adrenergic agonists have been proposed as treatment to offset muscle atrophy with such conditions as ageing[12], denervation[13, 14] and immobilization or unloading[6].

Different wasting disorders are characterized by a progressive diminution of the size of constituent muscle cells as a result of voluminous decline in their dimensions affecting not only contractile and related functions but also disturbing metabolic efficiency of tissue. The precise mechanism of the action of different ß-adrenoceptor agonists in ameliorating denervation atrophy is still not understood though an increased protein synthetic rate coupled with a decreased degradation rate is largely held responsible for growth promoting effects[2,15,16]. Aim of the present study is to investigate reparative and regenerative changes at histopathological levels accompanying ß-agonist induced amelioration of denervation atrophy of rat gastrocnemius. More specifically, changes in nuclear morphology, nuclear and fiber dynamics accompanying ß-adrenoceptor agonist induced

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reversal of denervation atrophy in rat gastrocnemius muscle have been investigated.

Materials and Methods

Animals—The study was conducted on healthy adult male albino rats showing no signs of morbidity. The animals were maintained in the animal house of the department under suitable hygienic conditions providing daylight (16 hr) and temperature (20°±2°C). Animals were maintained in the animal house as per the guidelines of an Institutional Animals Ethics Committee. Rats were divided into six groups. Rats in the first group were normal innervated controls. Second group included rats that received either clenbuterol (1.2 mg/kg body weight) or isoproterenol (2 mg/kg body weight). Rats in third group received butoxamine alongwith either clenbuterol or isoproterenol at the same dose rate. Fourth group included denervated rats. Fifth group had denervated rats, which received either clenbuterol or isoproterenol. Rats in the sixth group received butoxamine in addition to either of the agonist clenbuterol or isoproterenol similar to third group. Denervation procedure has been described elsewhere[17].

Administration of drugs—Clenbuterol (1.2 mg/kg body weight) and isoproterenol (2 mg/kg weight) obtained from Sigma Aldrich, USA were administered to rats orally. Treatment of drugs began one day prior to denervation and continued till day 7 following denervation. β-adrenergic agonists were dissolved in double distilled water to get a stock solution of 1 mg/ml. Further dilutions were made at the time of treatment keeping in view the weekly weight record of the animals. The drug treated rats were killed on day 3, 7, 15, and 30. Age matched control rats were also killed on these days. Gastrocnemius muscle was removed and immediately processed for histopathological study. Paraffin embedded muscle sections (10 μm thick) were stained with hematoxylin eosin, examined under a light microscope and then photographed.

Nuclear dynamics studies—(i) Number of muscle nuclei/fiber cross-section: Total number of muscle nuclei per fiber cross section were estimated by direct counting of nuclei in enlarged photomicrographic prints. Four rats from each group were killed and equal number of gastrocnemii included in these measurements. At least 50-100 fibres were randomly selected from each muscle. Mean muscle nuclei/fiber cross section were calculated and standard error of mean calculated.

(ii) Number of muscle nuclei/mm fiber length: Haematoxylin eosin stained transverse muscle sections (10 μm thick) were employed for quantitative estimation of number of muscle nuclei per mm length of muscle fiber. Number of such myonuclei was calculated according to an equation of Schmalbruch and Lewis[18]. A total of 75-100 myonuclei were included for mean length as required in the equation.

$$X = N.1000\ (L+D)-1$$

where X = number of muscle nuclei/fiber cross section as calculated as above (i),
L = mean length of muscle nuclei (in μm), and
D = thickness of muscle section

(iii) Fiber diameter: Fresh frozen cryostat cut sections (10 μm) were employed for the determination of fiber diameter. The oculomicrometer fixed in the eye lens was calibrated against the stage micrometer and thereafter the muscle section was observed under it. The mean fiber diameter was calculated by including all principal fibre categories. An equal number of fast twitch glycolytic (white), slow twitch oxidative (red) and slow twitch oxidative glycolytic (intermediate) fibers were employed for the calculation of mean fiber diameters.

Results

Microscopic examination of hematoxylin stained muscle sections from normal innervated rats exhibited a conventional heterogeneous fiber population discernible on the basis of size. The entire fiber population comprised roughly oval or polygonal cells in transverse section (Fig. 1a). Constituent muscle cells displayed basophilic nuclei predominantly sub-sarcolemmal in disposition. At low magnification these muscle cell nuclei represented minute hematoxylin stained specs or dots. Intra nuclear details, however, become clear once these nuclei were examined under higher magnification. Such nuclei were vesicular with granular nucleoplasm. A distinct nucleolus was also discernible within the nucleoplasm (Fig. 1b). Muscle cell nuclei can be further grouped into two sub types: (i) oval, cylindrical, biconcave nuclei with an active granular cytoplasm and (ii) bilaterally compressed elongated nuclei with hyperactive condensed chromatin. Histological examination also revealed a sparse distribution of

interfibrillar or extra fibrillar nuclei occasionally associated with streaks of polymorphonuclear leukocytes (PMNL) especially in regions where capillaries are distributed.

Administration of ß-adrenoceptor agonists, clenbuterol and isoproterenol hydrochloride to normal innervated rats induced hypertrophy of a selected cell population in gastrocnemius muscle in the beginning (Fig. 2a). Such cells were commonly observed in tissue sections suggesting an onset of a stepped-up biosynthesis in the tissue. Muscle nuclei from treated rats exhibited an increase in their size and showed active granular nucleoplasmic distribution (Fig. 2b). Even bilaterally compressed nuclei revealed granular basophilia. Bean shaped oval or ovoid muscle nuclei tend to show either an end-to-end or a side-to-side polar alignment (Fig. 2c). With an increase in post treatment period, vesicular nature of nuclei was lost and these instead turned into elongated, extremely hyperactive bilaterally compressed rods (Fig. 2d).

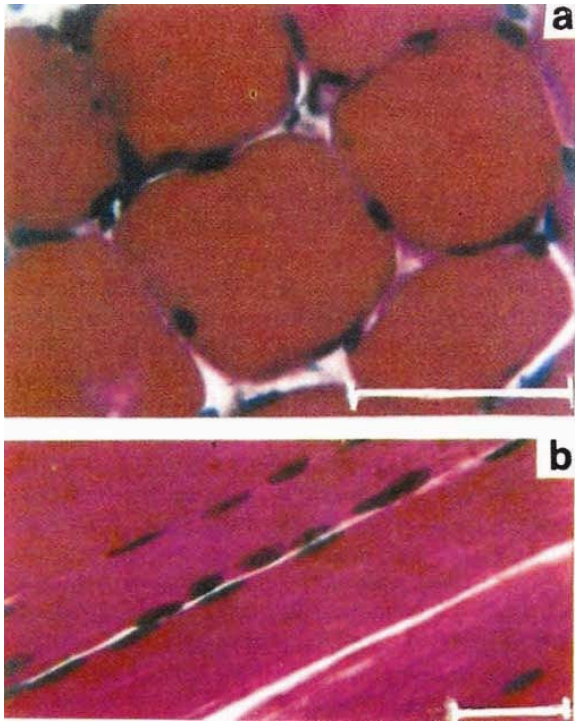

Fig. 1—Transverse section of rat gastrocnemius exhibiting constituent muscle cells with myonuclei occupying subsarcolemmal dispositions (a). Interfibrillar nuclei are also commonly seen (arrow). Intranuclear details are visible in longitudinal sections (b). Bar=50 µm

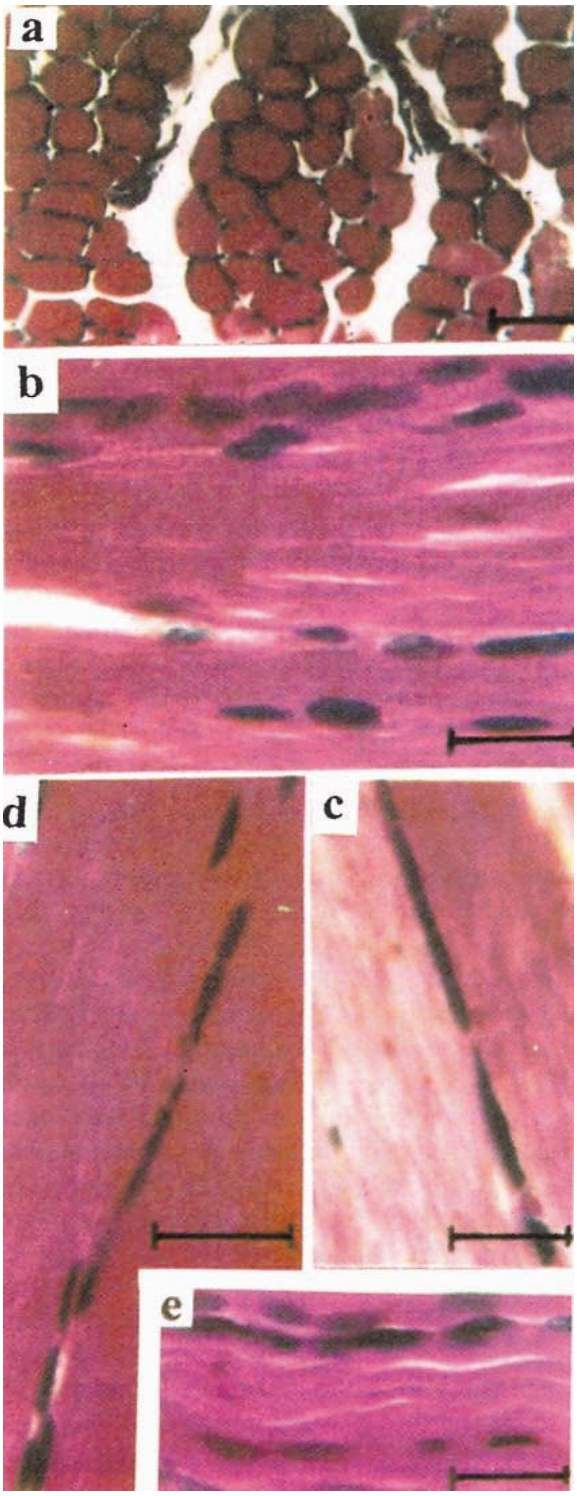

Fig. 2—Isoproterenol hydrochloride administration to normal innervated rats results in hypertrophy of fibers (a). Nuclei undergo enlargement in their size and display prominent nucleoli and euchromatin (b, e).Other notable features included end-to-end or a polar association of nuclei into unusually elongated nuclear aggregates (c,d). Bar=50 µm

Many such muscle nuclei make an end-to-end alignment. Such nuclear aggregates representing post fusion nuclear complexes were typically observed along sub sarcolemmal regions of treated cells in longitudinal sections (Fig. 2e).

Denervation caused marked anatomical changes in the organization of constituent muscle fibres. Effects of loss of neural supply begin with changes in the outlines of affected muscle fibres (Fig. 3a). Thereafter, a progressive myodegeneration ensued in affected muscle cells (Fig. 3b). Associated with these anatomical changes, invasion of the tissue by polymorphonuclear leukocytes was conspicuously noticed. Muscle sections from a denervated gastrocnemius exhibited atrophy of constituent muscle fibers and these fibers showed infiltration of polymorphonuclear leukocytes (Fig. 3a). By 15thday postdenervation, extensive myodegeneration on affected fibres was common (Fig. 3c). Cells showing incorporated PMNL were observed undergoing necrosis. These myodegenerating areas were overladen with lymphoid cell population and such tissue sections were almost completely hydrolyzed or instead replaced by lymphoid tissue (Fig. 3d). Muscle cell nuclei although present in large number, demonstrated remarkable alterations in their morphology. Vesicular nature of nucleoplasm was lost, nuclei appeared pyknotic and exhibited variable shapes ranging from condensed rods to curved, angular and dot like other crescent structures (Fig. 3b). Denervation thus resulted in a considerable loss of the structural and functional organization of the constituent muscle cells.

Administration of ß-adrenoceptor agonist clenbuterol to denervated rats not only inhibits the muscle atrophy and myofibrillar degeneration but also tends to restore tissue towards a state characteristic to normal innervated tissue. Administration of ß-agonist began one day prior to denervation thus ensuring its circulation even before tissue lost its neural control and continued for subsequent seven days. The first seven days witnessed hardly any recovery and/or regeneration in denervated gastrocnemius. However, histological signs of regeneration became noticeable

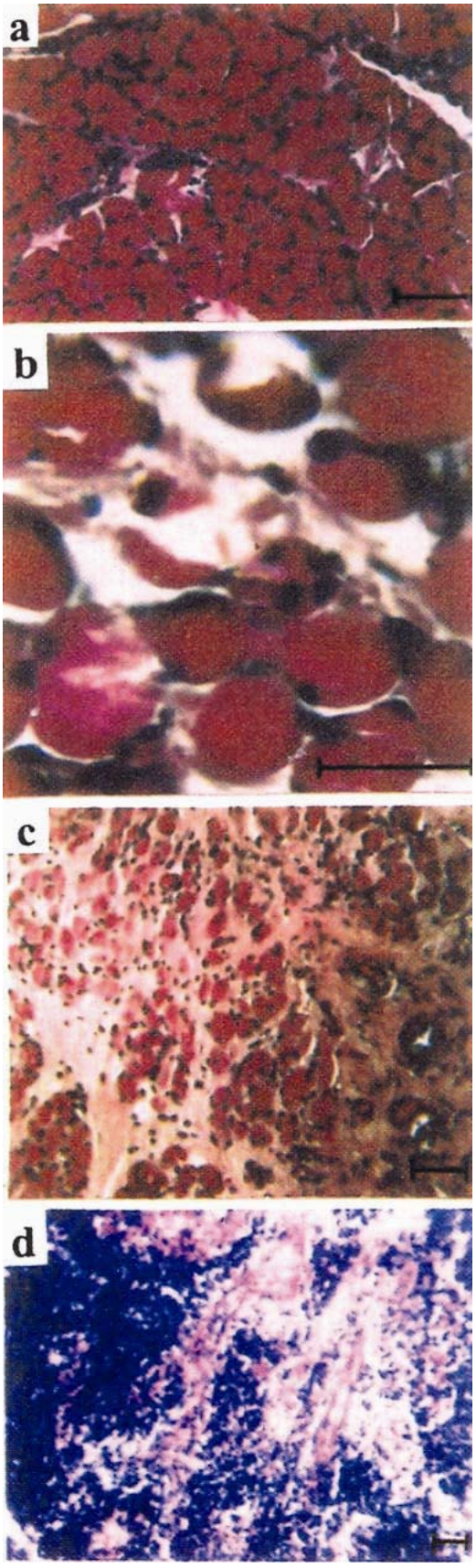

Fig. 3—Denervation of rat gastrocnemius results in altered fiber outlines and an infiltration of tissue by polymorphonuclear leucocytes-PMNL (a) and subsequent myofibrillar degeneration (b). Certain areas in tissue sections demonstrate extreme myodegeneration (c) that are evenly replaced by lymphoidal cells (d). Bar=50 µm

on day 15 in treated denervated gastrocnemius. These included (i) appearance of central muscle nuclei invariably in large population of affected muscle cells and emergence of unusually large hypertrophying cells amongst atrophying cell population (Fig. 4a). Both isoproterenol and clenbuterol were equally competent in bringing this qualitative improvement in the cellular organization of denervated muscle (Fig. 4a and b). Many cells remained invariably associated with infiltrating PMNL. Profuse streaks of PMNL were observed surrounding affected muscle fibers (Fig. 4c). Myo-degenerating regions were almost completely invaded by lymphoid tissue. The muscle tissue in such regions was in a complete lytic state and exhibited bits of tissue remnants amongst lymphoid cell population (Fig. 4d and e). Fragmented lumps of tissue can also be seen in the vicinity of many degenerating muscle cells.

Simultaneous administration of ß-receptor antagonist, butoxamine to isoproterenol and clenbuterol treated denervated rats confirmed an ability of ß-receptor agonists to ameliorate denervation atrophy. Muscle fibers in denervated gastrocnemius muscle from isoproterenol treated rats

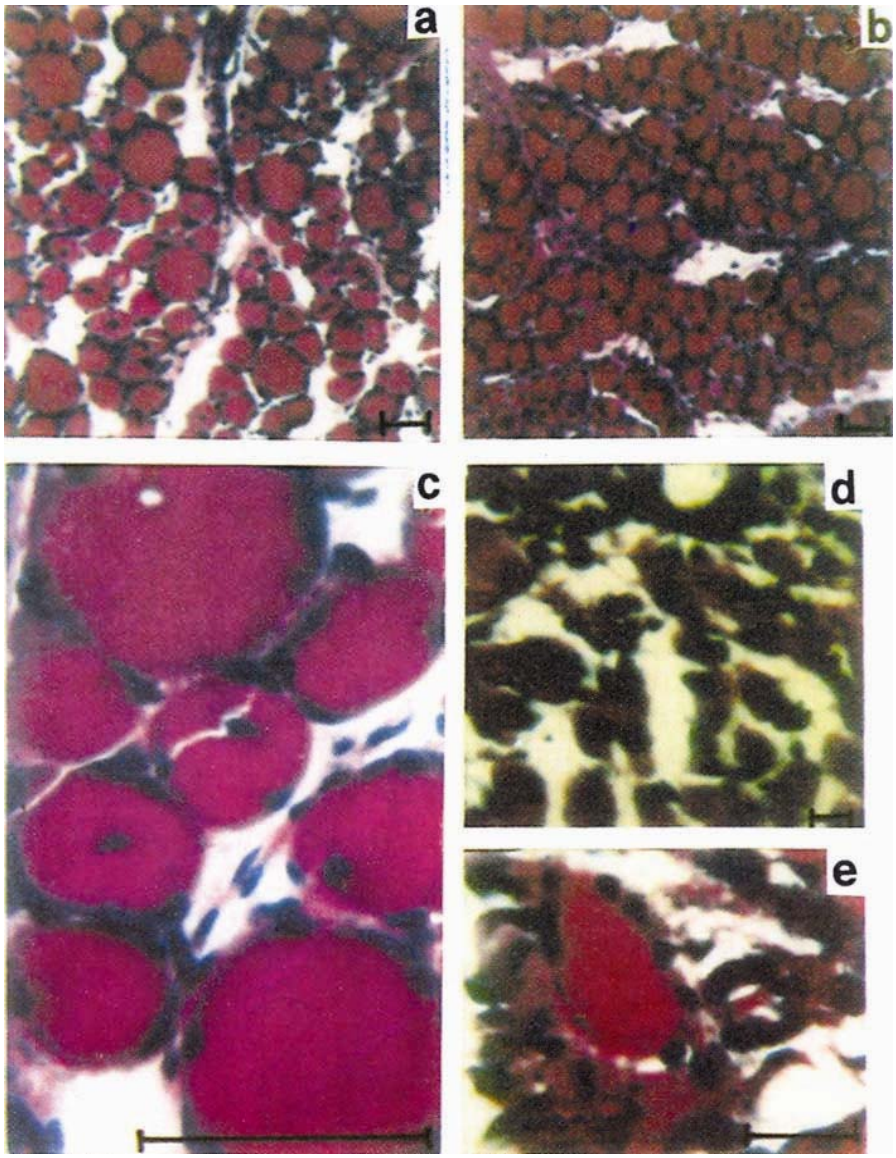

Fig. 4—Administration of isoproterenol and clenbuterol to denervated rat gastrocnemius results in hypertrophy of select cell population (a, b). Both atrophied and hypertrophied cells continue to show PMNL infiltration (c). Myodegeneration continues in some parts of sections (d,e). Bar=50 µm

that received butoxamine, surprisingly exhibited a near normal histological appearance (Fig. 5a). However, these fibers continued to display disfigured outlines and infiltration by PMNL was weak. Muscle nuclei in longitudinal sections were considerably enlarged, hyperactive with an appreciable amount of euchromatin in the form of a granular nucleoplasm (Fig. 5b and c). End-to-end nuclear alignment characteristic to normal innervated muscle treated with ß-agonists, supported continuous regeneration process (Fig. 5c). Butoxamine at the concentration employed thus proved relatively weak in inhibiting isoproterenol induced amelioration of denervation atrophy. On the other hand, denervated gastrocnemius from butoxamine and clenbuterol treated rats invariably showed histological organization parallel to denervated tissue in absence of ß-adrenoceptor agonists (Fig. 6a and b). Tissue sections from these preparations continued to exhibit an extensive myodegeneration (Fig. 6a) as characteristic to denervation atrophy. Cells displayed variable but irregular shapes and invariably demonstrate an incorporation of PMNL in them. Individual fibers were surrounded and overladen with numerous encircling PMNL (Fig. 6b). The myofibrillar degeneration continued even beyond day 30. Abundant blood cells encircling and engulfing the muscle cells were commonly observed (Fig. 6c). No muscle cells with central muscle cell nuclei were visible.

Dynamics of nuclei and their alterations in presence of ß-adrenoceptor agonists, clenbuterol and isoproterenol—Muscle sections from different tissues viz., normal innervated ± ß-agonists, and denervated gastrocnemius ± ß-agonists were photographed and muscle nuclei/fiber cross section counted (Fig. 7). Administration of clenbuterol, a ß-adrenoceptor agonist to normal innervated rats resulted in a significant increase on day 7. This increase in muscle nuclei continued till day 30. A similar increase in the number of muscle cell nuclei/fiber cross section was noticed with isoproterenol hydrochloride administration to normal innervated rats on day 7. Increase in muscle nuclei as a result of ß-agonist application was confirmed when butoxamine, a ß-adrenoceptor antagonist was also simultaneously administered to animals along with ß-adrenergic agonists. Butoxamine induced change in muscle nuclei was, however, convincing only in rats, which had received isoproterenol hydrochloride.

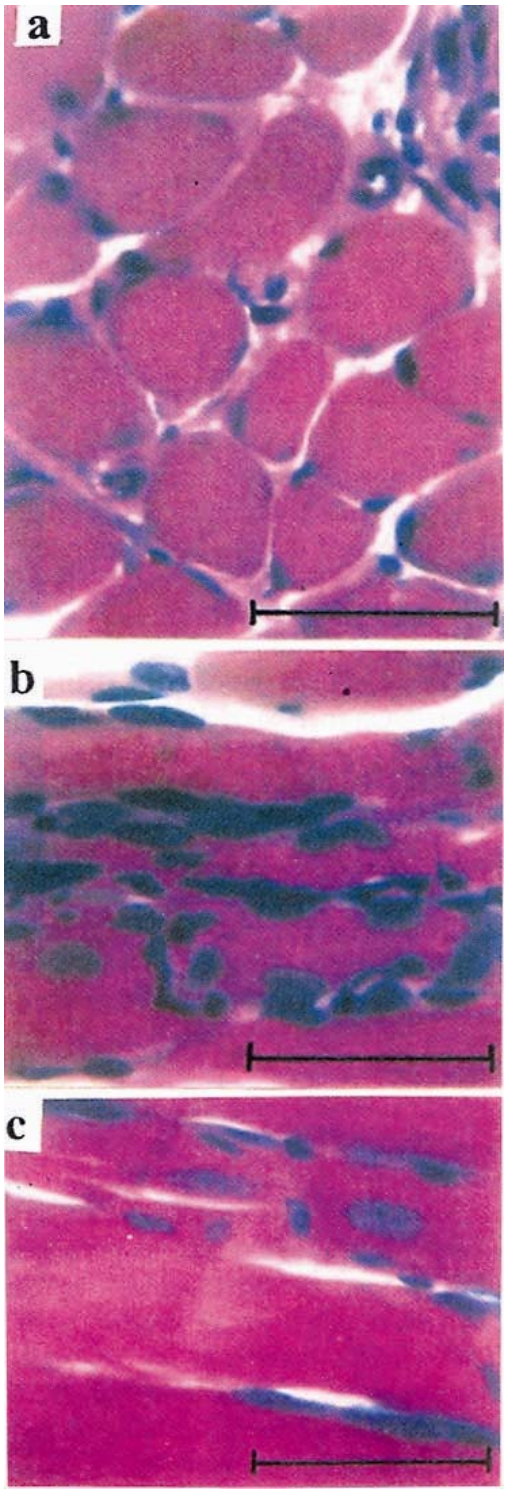

Fig. 5—Simultaneous administration of butoxamine, a ß-adrenoceptor antagonist to denervated rats which received isoproterenol hydrochloride confirmed an alleviation of atrophy. Muscle fibers tend to recover towards near normal outlines (a). Muscle nuclei are exceptionally large in size with bizarre shapes and display very active euchromatin (b,c). Bar=50 µm

Administration of ß-receptor antagonist, butoxamine to clenbuterol treated normal innervated rats had no effect so far as number of muscle nuclei/fiber cross section is concerned.

Denervation typically resulted in a significant increase in number of muscle nuclei/fiber cross section in rat gastrocnemius. The stimulation in number of muscle nuclei in denervated rat gastrocnemius was, however, inhibited in presence of clenbuterol but isoproterenol hydrochloride was unable to do so. That the effects of different ß-adrenoceptor agonists manifest through neural axis, was further confirmed from the measurement of muscle nuclei in denervated rats which received antagonist alongwith the ß-agonists. Butoxamine significantly inhibited stimulated muscle nuclear number in denervated muscle from isoproterenol and clenbuterol treated animals.

ß-Agonist induced alterations in number of muscle nuclei/mm length of muscle fiber—Normal innervated rat gastrocnemius muscle demonstrated 126 ± 17 muscle nuclei per mm length of muscle fiber (Table 1). This number was further stimulated in presence of clenbuterol and isoproterenol hydrochloride. Administration of butoxamine, a ß-receptor antagonist to these rats, however, attenuated this increase in nuclear number/mm length of muscle fiber and the values dropped to subnormal levels.

Measurements of the number of muscle nuclei further revealed a denervation induced significant decline. The denervated rat gastrocnemius exhibited as low as 82 ± 09 muscle nuclei/mm muscle fiber length, a figure as low as 65 % when compared with the corresponding figure from normal innervated muscle. Treatment of denervated muscle with ß receptor agonist, clenbuterol and isoproterenol hydrochloride resulted in a partial restoration of original nuclear number. These values are as high as 94% and 90 % of the number characteristic to normal innervated muscle. Butoxamine application to denervated rats already receiving ß-agonists restored muscle nuclei number towards those of normal innervated muscle. The results suggest that administration of ß-adrenergic agonists caused (i) an increase in the muscle nuclei in normal innervated muscle and (ii) tend to restore declining number of muscle nuclei from denervated muscle. It implies therefore that drugs act via neural axis, a view already shared by many.

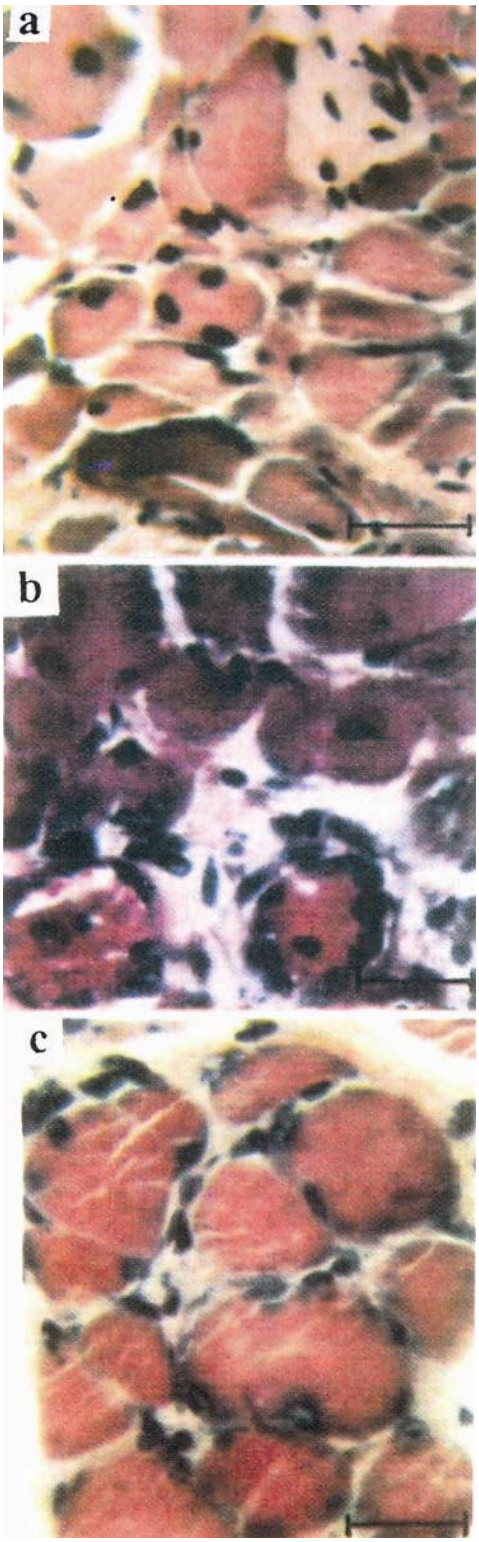

Fig. 6—Butoxamine treatment to denervated rats which received clenbuterol failed to ameliorate denervation induced atrophy in gastrocnemius. Muscle fibers continue to demonstrate altered outlines (a) and degenerating fibers invariably demonstrate leukocyte infiltration (b). Muscle nuclei are pycnotic (c). Bar=50 µm

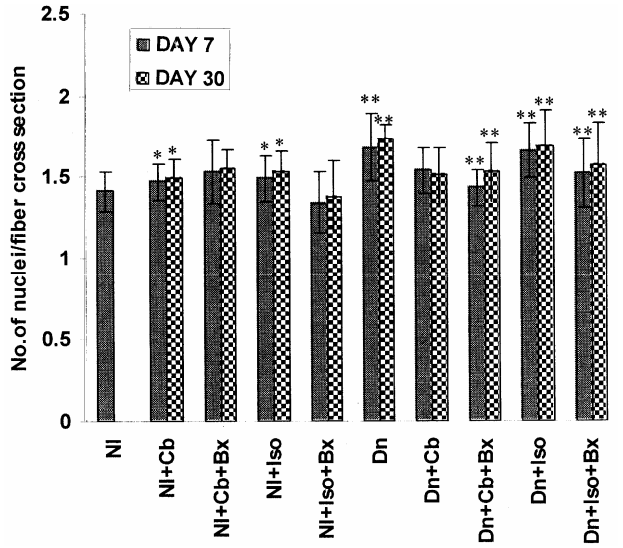

Fig. 7—Changes in number of muscle nuclei/fiber cross section in normal innervated (NI) and denervated (Dn) rat gastrocnemius muscle and the alterations following treatment with ß-adrenoceptor agonists, clenbuterol (Cb) and isoproterenol hydrochloride (Iso). Butoxamine (Bx), a ß-receptor antagonist was used to confirm stimulations in numbers. Values were significant at * $P<0.05$ and ** $P<0.01$; n=4 each.

ß-Agonist induced changes in fiber dimensions—Normal innervated rat gastrocnemius exhibited a mean fiber diameter of 30.6 ± 0.91 μm (Table 1). Hypertrophic effect of ß-agonist was established from an increased mean fiber dimension from gastrocnemius muscle of the rats treated with isoproterenol and clenbuterol. Clenbuterol was relatively more effective in inducing hypertrophy in comparison to isoproterenol. ß-agonist induced growth promoting hypertrophic effect in gastrocnemius muscle was also confirmed from normal innervated animals that received both ß agonists and antagonists together. The mean fiber diameter in such muscle dropped to a value close to normal innervated muscle. Denervation resulted in a marked significant reduction in mean fiber diameter. Voluminous decline in fiber dimension suggests an onset of fiber atrophy, which became well established by day 7. However, muscle atrophy was well inhibited in denervated rats that received either isoproterenol hydrochloride or clenbuterol. Mean fiber diameters of such denervated rat gastrocnemii rose considerably in presence of clenbuterol and isoproterenol when compared to denervated gastrocnemius. Effects of ß-agonists in reversing or attenuating fiber atrophy were partly inhibited when denervated rats also received butoxamine along with clenbuterol and isoproterenol. A slight decrease in the mean fiber diameter became noticeable in the denervated gastrocnemius treated with isoproterenol and butoxamine in comparison to denervated gastrocnemius.

Table 1—Clenbuterol (Cb) and isoproterenol hydrochloride (Iso) {± butoxamine hydrochloride (Bx)} induced changes in muscle nuclei/mm fiber length and fiber diameter of rat gastrocnemius muscle from normal innervated (NI) and denervated (Dn) rats on day 7

[Values are mean ± SE from 5 observations in each group]

Groups	Muscle nuclei/mm fiber length	Fiber diameter (μm)
NI	126±17	30.60±0.91
NI+Cb	139±18*	34.56±1.89*
NI+Cb+Bx	130±21**	31.04±1.11
NI+Iso	145±22*	32.20±0.81
NI+Iso+Bx	126±18**	26.40±0.12
Dn	82±09	20.8±0.85**
Dn+Cb	119±14	25.80±2.14
Dn+Cb+Bx	116±21	27.50±0.42
Dn+Iso	113±19	26.23±2.11
Dn+Iso+Bx	124±15	25.80±0.62

P values: *<0.05; ** < 0.01

Discussion

ß-adrenoceptor agonists successfully ameliorated atrophic condition of denervated skeletal muscle by reorganizing the tissue both structurally and functionally. Restoration of structural organization of muscle cells and nuclei almost characteristic to normal innervated muscle cells and changes in phenotypic expression of denervated skeletal muscle provide testimony to this. In normal innervated muscle the drugs are capable of inducing muscle fiber hypertrophy in general which is established from an increase in the mean fiber diameters irrespective of the category these belong to. Percent increase in the fiber diameters also corresponds with the muscle weights in different vertebrates[19,20]. Fiber hypertrophy is therefore, a common anabolic effect of ß-adrenoceptor agonists[21] in addition to changes produced in protein, RNA and DNA accretion[15,22-24]. It remains the most striking response of normal innervated skeletal muscle to ß-adrenoceptor agonists treatment as no evidence as yet is available about drug induced increase either in satellite cell proliferation or myotubes generation in the tissue in normal innervated condition. Mean fiber dimensions of clenbuterol and isoproterenol treated rat gastrocnemius muscle show significant variation. That these variations are ß-agonists specific is

confirmed from near restoration of original mean dimensions in presence of butoxamine, a ß-adrenoceptor antagonist.

ß-adrenergic agonists specifically promote protein accretion both in innervated and denervated skeletal muscle[24]. Predictably therefore, growth stimulating effects of ß-agonists have been demonstrated in conditions characterized by atrophy of constituent cells such as ageing[12], and denervation atrophy[6,13]. These drugs can oppose muscle weaknesses and hence are proposed as valuable adjunct to different wasting disorders[6]. Quantitative data obtained on fiber diameters suggested ß-adrenoceptor activation resulted in an amelioration of atrophic state. Results were successively confirmed by administration of butoxamine. Since atrophy resulted from an abrogated neural regulation and was reversed in presence of ß-adrenoceptor agonists, the drugs must therefore be acting via neural axis[14]. An analysis of fiber dimensions shows that well established atrophy in denervated tissue was inhibited in presence of ß-receptor agonist. Although precise mechanism responsible for the action of different ß-agonists remains unclear, it is postulated that these drugs may be mimicking, interacting or modulating innervation functions. The results of present study provide sufficient evidence in favour of such a mechanism.

An examination of tissue sections of denervated muscle in the present study exhibited that ß-adrenoceptor agonists induced effects that were multifarious. While a select population of fibers exhibited hypertrophy, others especially those undergoing atrophy or degeneration responded by inhibiting an infiltration by polymorphonuclear leukocytes. Hypertrophy appears to be a consistent feature in denervated muscle, which as discussed earlier was documented from different measurements on fiber diameters. Myofiber regeneration and repair is important in diseased or injured muscles[25]. No evidence other than cell enlargement or myotube formation in tissue as a result of ß-agonists' administration is available. Hyperplasia is also ruled out to be a factor contributing to drugs induced amelioration of denervation atrophy. Recently, Katoch and Sharma[26] have documented an emergence of *de novo* cells in denervated gastrocnemius muscle following the administration of clenbuterol to adult chicks. Whether these differences are species specific or age related cannot be confirmed at this point. Sexually mature adult denervated chicks were used in the study[26]. Emergence of cells was consistently documented in the denervated gastrocnemius muscle in the presence of clenbuterol. The cells undergo growth and ultimately become indistinguishable from the rest of population. This is perhaps supported by parallel histological picture in denervated skeletal muscle. Denervation, for example, causes an initial proliferation of satellite cells[27] that ultimately declines. There is a sustained cellular proliferation in denervated mouse EDL[28] as confirmed from immediate spurt in ^{4}H-Thymidine incorporation in denervated muscle followed by disappearance. Myotube like fibers occur in some muscles like soleus of mice and rat, few weeks after denervation[29] which have been interpreted as signs of regeneration after loss of muscle fibers.

The reparative process that begins one week after drug administration was also confirmed from the appearance of central muscle cell nuclei in some parts of muscle sections. Appearance of these central muscle cell nuclei in affected muscle fibers denotes an onset of the process of regeneration. A progressive decline in necrotic fibers coupled with an accumulation of centrally nucleated muscle fibers adds strength to this possibility. Usually, diseased muscles including those from mdx mice lack an inherent capacity of regeneration[30]. Once injured, these tend to behave like normal one[31]. Muscle cell nuclei are generally peripherally placed prior to the onset of necrosis[32]. Following necrosis, regeneration occurs which is recognized by usual cytoplasmic basophilia and centrally placed euchromatic nuclei[33]. Centrally placed nuclei continue to persist during regeneration and can be employed as markers of such a reconstruction process[32, 34, 35].In limb gastrocnemius muscle, regeneration seems to produce a population of centrally nucleated fibres that are relatively resistant to necrosis.

Tissue growth is a consequence of an accelerated protein biosynthesis coupled with a decreased rate of catabolism. ß-adrenergic agonists induced amelioration of atrophic, dystrophic and other diseased states of muscles results following the tilting of equilibrium in favour of biosynthesis[15,24,36]. The observations are ably supported by studies on nucleic acid metabolisms as well[37]. Nuclei provide seat of nucleic acid synthesis and hence offer viable information on their association with growth related activities of the tissue. Since skeletal muscle is a multinucleate tissue, its growth results from

cumulative contribution of the entire nuclear component. Individual nuclei are likely to exert control over the proteins expressed in their immediate vicinity or in other words, each nucleus has its own domain. Thus, changes in nuclei could account for variable responses of tissue with regards to the synthetic activities. Muscle nuclei from treated rats exhibited features like vesicular granular nucleoplasm, enlarged oval and bean shaped hyperactive outlines as well as an increase in their number both per fiber cross section and per mm muscle fiber length. This pointed to an elevated biosynthetic activity in the tissue in presence of ß-adrenoceptor agonists. A conspicuous end-to-end alignment of enlarged hyperactive nuclei in treated tissue from normal innervated rat suggest onset of increased biosynthetic phase. There is a little information available on the participation of nuclei in different patho-physiological states including myonecrosis. A study conducted on xylotox (lignocaine hydrochloride) induced myonecrosis in mice[34] had demonstrated a characteristic association of unsually elongated subsarcolemmal nuclear chains as noticed in the present study also, accompanying skeletal muscle regeneration. Thus, an emergence of central muscle nuclei, hyperactive granular nucleoplasm and their association into long chains are some of the features that are typical of a regeneration and/or growth related reconstruction processes characteristic to skeletal muscle.

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