Laboratory Investigations

Properties of fibres, endplates and acetylcholine receptors in the diaphragm, masseter, laryngeal, abdominal and limb muscles in the goat

Chikwendu Ibebunjo DVM PhD,*
Coimbatore B. Srikant PhD,†
François Donati PhD MD FRCPC*‡

Purpose: *Although differences in fibre composition, fibre size or acetylcholine receptor (AChR) density between muscles have often been proposed to explain the unequal sensitivities of muscles to muscle relaxant drugs, it is not clear whether or how these parameters differ among muscles or are related to one another. In this study, several muscles were examined to determine the composition and cross-sectional area (CSA) of types I and II fibres, the surface area of their motor endplates (ESA), and their AChR density.*

Methods: *Biopsies from the thyroarytenoideus, cricoarytenoideus dorsalis, masseter, diaphragm, transversus abdominis, rectus abdominis, gastrocnemius and soleus muscles of goats were processed by muscle histochemistry and morphometry and the ESA:CSA ratio was computed. The number and density of AChRs per endplate were estimated by ^{125}I-α-bungarotoxin binding studies.*

Results: *The mean type I fibre composition (range: 0–100%), fibre diameter (28–50 μm) and the ESA:CSA ratio (0.27–1.01) differed among muscles ($P = 0.0001$), but there were no significant differences ($P > 0.05$) in the mean endplate size (577–725 μm^2), AChR number (6.6–14.5 × 10^6) or AChR density (8,900–22,300 μm^{-2}) probably because of marked individual variations. Fibre size increased and the ESA:CSA ratio decreased in the order laryngeal, diaphragm, jaw, limb and abdominal muscles.*

Conclusion: *It is concluded that between muscles fibre size varies more than endplate size or AChR number.*

Objectif: *Bien que la disparité de composition et de dimension des fibres et de densité des récepteurs acétylcholinergiques (AChR) entre les muscles ait été proposée comme explication pour l'inégalité possible de la sensibilité des muscles aux relaxants musculaires, on ne sait pas vraiment si elle existe et comment ces paramètres différent entre les muscles et quelle relation existe entre eux. Pour cette étude, plusieurs biopsie musculaires ont été examinées; elles provenaient des muscles thyroaryténoïdiens, cricoaryténoïdiens, dorsaux, masséters, diaphragmatiques, droits abdominaux, gastrocnémiens et soléaires de chèvres et ont été traitées par histométrie et morphométrie musculaires. Il s'agissait de déterminer la composi-*

Key words

MUSCLE, SKELETAL: cricoarytenoideus dorsalis, thyroarytenoideus, diaphragm, masseter, soleus, gastrocnemius, transversus abdominis, rectus abdominis: morphology;
NEUROMUSCULAR JUNCTION: acetylcholine receptor.

From the Departments of Anaesthesia* and Medicine†, Royal Victoria Hospital and McGill University, Montréal, Québec, Canada H3A 1A1.

‡*Present address*: Professor and Chairman, Department of Anesthesia, Hotel-Dieu Hospital and University of Montréal, Montréal, Canada.

Address correspondence to: Dr. C. Ibebunjo, Shriners Burns Institute Research Center, Bldg., 1400W, One Kendall Square, Cambridge, MA 02139, U.S.A.

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tion et la surface transversale des fibres (CSA) de type I et II, et la superficie de leurs plaques motrices (ESA). La rapport ESA:CSA a été calculé par ordinateur. Le nombre et la densité des AChR par plaque motrice a été estimé grâce à des études de liaison à la ^{125}I-α-bugarotoxin.

***Résultats**: La composition moyenne des fibres de type I (étendu 0–100%), le diamètre des fibres (28–50 μm) et la rapport ESA:CSA (0,27–1,01) différaient entre les muscles (P = 0.0001), mais il y avait pas de différences entre la dimension des plaques motrices (577–725 μm^2), le nombre (6,6–14,5 × 10^6) et la densité d'AChR (8900–22300 μm^{-2}) vraisemblablement à cause des variations individuelles marquées. La dimension des fibres augmentait et le rapprt ESA:CSA diminuait dans l'ordre suivant: larynx, diaphragme, mâchoire, membres et abdomen.*

***Conclusion**: Entre les muscles, la dimension des fibres varie plus que la dimension des plaques motrices et le nombre d'AChR.*

Striated muscles differ in their electrophysiology, biochemical and contractile properties, in blood perfusion and in the composition and size of their constituent fibre types.[1,2] They also differ in sensitivity to neuromuscular blocking drugs for reasons that are poorly understood.[3] Early experiments in cats found that the soleus and diaphragm muscles were more sensitive to d-tubocurarine and less so to decamethonium than the tibialis cranialis muscle. Since the soleus and diaphragm were considered red (slow-twitch) muscles and the tibialis cranialis a white (fast-twitch) muscle, it was concluded that muscle sensitivity is related to fibre type composition.[4] Differences between the sensitivity of the diaphragm and peripheral muscles have also been attributed to differences in muscle temperature,[5] while the observations that pathophysiological conditions that produced muscle atrophy or proliferation of acetylcholine receptors altered muscle sensitivity suggested a role for fibre size[6] and acetylcholine receptor distribution.[7] A more recent study found a positive correlation between fibre size and the duration of action of succinylcholine or vecuronium in four muscles in the anaesthetized goat.[8] Since relaxant drugs produce their effects by binding with acetylcholine receptors at the motor endplates, it is likely that fibre size is related to some characteristics of motor endplates and/or acetylcholine receptors. There is some evidence that within a given muscle, the size of the endplate varies directly with muscle fibre size,[9] but it is not known whether this relationship holds across muscles that differ in fibre size. Moreover, it is not known how the muscles of importance to anaesthetists, namely muscles of the larynx, diaphragm, abdominal wall, jaw and limbs in the same subject, differ in fibre type composition, fibre size, endplate size and acetylcholine receptor distribution.

The objective of this study was to determine whether there are systematic differences in the fibre type composition, fibre size, endplate size and acetylcholine receptor number or density among the diaphragm, masseter, laryngeal, abdominal and limb muscles in the goat that could contribute to explain the unequal effects of neuromuscular blocking drugs in these muscles.

Methods

Animals

Fourteen adult female goats of the Saanen breed were used for this study after approval by the Animal Care Sub-Committee of McGill University. Seven of the goats (age, 24.7 ± 13.5 months; body weight, 30.3 ± 11.5 kg) had been used for pharmacodynamic studies on succinylcholine chloride (Quelicin™, Abbott Laboratories Ltd, Montreal, Canada) and the other seven (age, 25.6 ± 2.3 months; body weight, 29.9 ± 2.4 kg) to study vecuronium bromide (Norcuron®, Organon Canada, West Hill, Ontario, Canada).[10]

Histological studies

When the evoked electromyographic response had recovered maximally in all muscles, the goats were sacrificed with an overdose of pentobarbitone. Muscle specimens were taken from the cricoarytenoideus dorsalis, thyroarytenoideus, costal diaphragm, transversus abdominis, rectus abdominis, soleus, gastrocnemius and masseter muscles. Specimens from the laryngeal muscles extended the entire muscle length but for other muscles they included the motor endplate region. The specimens were rapidly frozen in isopentane precooled to its freezing point in liquid nitrogen, and stored at −80°C until ready for sectioning or autoradiographic studies. Serial cross-sections, 10 μm thick, were cut at −25°C and stained for myosin adenosine triphosphatase (mATPase) activity after preincubation at pH 9.4, 4.3 and 4.2, and for oxidative enzyme (reduced nicotinamide adenine dinucleotide dehydrogenase–tetrazolium reductase, NADH-TR, or succinic dehydrogenase, SDH) capacity.[11] Fibres that stained weakly for mATPase at pH 9.4 and strongly for oxidative enzymes were classified as type I (slow-twitch) while fibres that stained strongly for mATPase at pH 9.4 and weakly for oxidative enzymes were classified as type II (fast-twtich).[11] Under camera-lucida microscopy, at least 1000 fibres in each section were differentially counted, and the population of each type expressed as a percentage of the total. In each section, the cross-sectional area (CSA, μm^2) of 100–200 of each fibre type was mea-

sured by computerized planimetry (JAVA®, Jandel Scientific, USA). To ensure that the results obtained were accurate and representative of the muscle, only transversely cut sections were used, and the entire section was scanned in a zigzag manner and 6–10 fields randomly selected for differential fibre counting or area measurement.

Acetylcholine receptor studies

The number of acetylcholine receptors per endplate was estimated by ^{125}I-α-bungarotoxin (^{125}I-αBTX) binding studies on single muscle fibres as described previously.[12] Briefly, fresh or frozen 2–3 mm thin strips cut along the muscle length were incubated at room temperature for three hours in Krebs-Ringer solution containing 1.0 $\mu g \cdot ml^{-1}$ ^{125}I-αBTX (15 $\mu Ci \cdot \mu g^{-1}$; Du Pont, Canada), rinsed thoroughly in several changes of Krebs-Ringer solution to remove all unbound toxin, fixed for six hours in 4% glutaraldehyde in Millonig buffer (0.12M sodium phosphate monobasic, 0.07M sodium hydroxide and 5 $g \cdot L^{-1}$ glucose), and dissociated into single fibres using a Polytron homogenizer (Brinkman PCU-11, probe PT 10/35) at low speed.[12] The dissociated fibres were incubated at 37°C for 90 min in an acetylthiocholine medium[1] to stain and identify fibre segments bearing endplates. The fibres were then washed in several changes of buffer and stored at 4°C in buffer containing 0.1 $\mu g \cdot L^{-1}$ sodium azide. Under a dissecting microscope, at ×30 magnification, each fibre with an endplate was cut into pairs of segments of identical lengths (~1 mm), and sorted into segments with (junctional) or without (extrajunctional) endplates. The amount of radioactivity bound to a known number (usually 30–50) of junctional or extrajunctional segments was quantitated using a gamma counter (1272 Clinigamma, LKB Wallac, Finland). The number of αBTX molecules corresponding to one gamma count per minute was estimated from a curve relating the number of counts per minute (corrected for counter efficiency) produced by a known quantity of ^{125}I-αBTX (2.5×10^{-4} μg to 7.5×10^{-2} μg). One count per minute corresponded to 2.54×10^6 αBTX molecules. The difference between counts per minute bound to junctional and extrajunctional segments was computed, divided by the number of fibre segments, corrected for radioactive decay and multiplied by the number of αBTX sites corresponding to one count per minute to obtain an estimate of the number of αBTX binding sites per endplate.[14] This gamma count method of estimating the number of junctional acetylcholine receptors has been shown to yield estimates comparable with those obtained by electron microscopic (EM) autoradiography.[15]

The fibre segments were then mounted on microscope slides with an aqueous mountant (Farrant's Medium, BDH Inc.). Under camera-lucida microscopy the smallest smooth perimeter around each endplate presented *en face* was traced to obtain the endplate surface area. The diameter of the corresponding fibre was also measured. The endplate area measurement was not corrected for fibre surface concavity.[12] The mean density of acetylcholine receptors at the endplate was estimated by dividing the number of αBTX binding sites per endplate by the mean area of the endplate for that muscle.

Statistical analysis

For each muscle in each goat, fibre type composition (%), mean fibre cross-sectional area (μm^2), mean endplate surface area (μm^2), the number and density (μm^{-2}) of αBTX binding sites per endplate were obtained, and the mean (±SE) values of each variable for all goats computed. Differences in these variables between muscles were investigated by one-way ANOVA and the Scheffe test. In the case of the number or density of αBTX binding sites, because there were marked variations among goats, estimates for each of the eight muscles were normalized relative to the gastrocnemius muscle in the same goat and the normalized values compared. Although the normalized values were used for statistical analysis, the absolute number and density of αBTX binding sites are presented (Table II). The ratio of the endplate surface area to fibre cross-sectional area was calculated for each muscle and compared by one-way ANOVA. Linear regression analysis was used to investigate whether and how fibre composition, fibre cross-sectional area, endplate area, the endplate area to fibre area ratio, acetylcholine receptor number and receptor density may be related to one another. Statistical analyses were performed using StatView 512+® (version 1.2, BrainPower, Inc) on a Macintosh computer. Results are presented as mean ± SEM. $P \leq 0.05$ was considered statistically significant.

Results

Muscle fibre characteristics

The mATPase reaction at pH 9.4 differentiated types I (light) and II (dark) fibres. In all muscles except the masseter, this mATPase reaction was reversed following preincubation at pH 4.2–4.3 so that type I fibres stained dark and type II fibres light. Occasionally two subtypes of type II fibres, light (IIA) or intermediate (IIB), were differentiated following acidic preincubation. However, because the latter was not consistently obtained, fibre classification was limited to type I or II only. Fibres in the masseter were designated IM (M for masseter) to

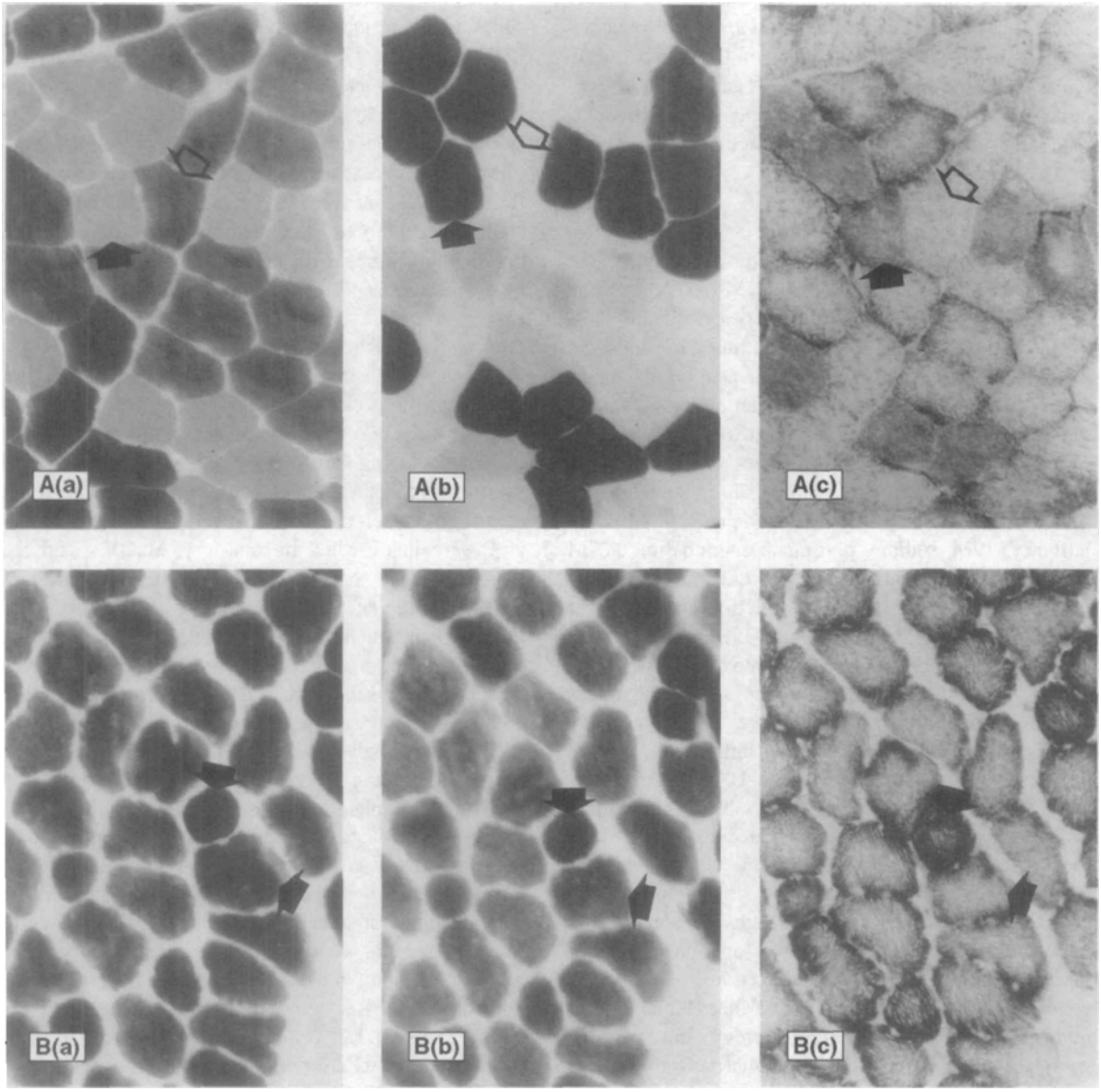


FIGURE 1 Photomicrographs of serial sections stained for mATPase activity after preincubation at pH 9.4 (a) and 4.3 (b), and for NADH-TR activity (c). (A) gastrocnemius; (B) masseter muscles (Same magnification). Note that type I fibres in the gastrocnemius stained weakly (filled arrow) and type II fibres strongly (open arrow) for mATPase at pH 9.4 and that this staining pattern was reversed following preincubation at pH 4.3. These types I and II fibres stained strongly and weakly to moderately for NADH-TR activity, respectively. In contrast, in the masseter, fibre mATPase activity at pH 9.4 was not reversed by preincubation at pH 4.3. These fibres had moderate to strong NADH-TR activity and were classified as type IM (filled arrows).

reflect that, unlike in other muscles, their mATPase activity was not reversed by preincubation at acidic pH. Oxidative enzyme (SDH or NADH-TR) capacity was high in type I fibres in all muscles except the soleus, moderate or low in type II fibres in the diaphragm, abdominal and limb muscles, and moderate or high in type II fibres in laryngeal muscles (Figure 1).

Type I fibre composition differed among muscles ranging from 10% in the thyroarytenoideus to 100% in the masseter and soleus (F-test = 0.0001; Table I). Fibre type composition also varied, albeit to a lesser extent, between the same muscle across goats.

The mean fibre cross-sectional area (CSA) differed among muscles ranging from 762 μm^2 to 2495 μm^2 in

TABLE I The mean (± SE) of the composition (%) and cross-sectional area (CSA μm²) of types I and II fibres in eight muscles in the goat.

Muscle	*Type I fibre composition (%)*	*Type I fibre CSA (μm²)*	*Type II fibre CSA (μm²)*	*Types I & II fibre CSA (μm²)*
1 Thyroarytenoideus	10.3 ± 2.6	520 ± 57	851 ± 73	762 ± 58
2 Cricoarytenoideus dorsalis	43.7 ± 5.7	658 ± 29	988 ± 46	835 ± 32
3 Diaphragm	58.0 ± 1.7	1502 ± 84	1548 ± 110	1516 ± 87
4 Masseter*	100.0 ± 0.0	1694 ± 86	–	1694 ± 86
5 Gastrocnemius.	32.5 ± 1.7	1565 ± 81	1962 ± 69	1812 ± 69
6 Soleus	100.0 ± 0.0	1912 ± 103	–	1912 ± 103
7 Transversus abdominis	35.5 ± 0.8	1531 ± 60	2109 ± 117	1933 ± 81
8 Rectus abdominis	32.3 ± 1.1	2311 ± 114	2615 ± 197	2495 ± 159
F-test	0.0001	0.0001	0.0001	0.0001

*Fibres in the masseter are of the special IM type (see text).

TABLE II The mean (± SE) endplate surface area (ESA, μm²), ratio of ESA to fibre cross-sectional area (ESA:CSA), and number and density of α-bungarotoxin (α-BTX) binding sites per endplate.

Muscle	*ESA (μm²)*	*ESA:CSA ratio*	*α-BTX sites per endplate (×10⁶)*	*α-BTX sites per μm² (×10³)*
1 Thyroarytenoideus	727 ± 27	1.01 ± 0.12*	10.8 ± 1.2	13.8 ± 1.4
2 Cricoarytenoideus dorsalis	689 ± 24	0.82 ± 0.03*	10.3 ± 1.1	14.6 ± 1.6
3 Diaphragm	625 ± 39	0.41 ± 0.04	10.6 ± 1.9	16.4 ± 2.8
4 Masseter	607 ± 19	0.38 ± 0.02	6.6 ± 1.3	8.9 ± 1.2
5 Gastrocnemius	579 ± 43	0.32 ± 0.03	8.0 ± 1.8	14.8 ± 3.6
6 Soleus	633 ± 29	0.33 ± 0.03	10.7 ± 1.8	14.7 ± 2.3
7 Transversus abdominis	656 ± 46	0.34 ± 0.03	14.5 ± 1.9	22.3 ± 3.5
8 Rectus abdominis	634 ± 30	0.27 ± 0.03	10.5 ± 1.4	16.4 ± 2.5
F-test	0.065	0.0001	0.084	0.043†

*$P < 0.05$ between laryngeal muscles and other muscles.
†$P > 0.05$ (Scheffe test).

the thyroarytenoideus and rectus abdominis, corresponding to approximately 30–55 μm in diameter, respectively (F-test = 0.0001; Table I). Mean fibre CSA increased in the order laryngeal, diaphragm, masseter, limb and abdominal muscles and there were considerable individual variations. Within muscles, type II fibres had larger mean CSA and coefficient of variation than type I fibres (Table I).

Motor endplate characteristics

The shape of motor endplates varied widely between fibres from within and between muscles ranging from round to elliptical to very irregular. In some fibres from laryngeal muscles, the endplate enveloped most of the fibre circumference, and where groups of two or more of such fibres had not been dissociated the endplates of the adjacent fibres were often in confluence. In contrast to these laryngeal fibres, fibres from limb and abdominal muscles had motor endplates that occupied only a small portion of the fibre circumference and did not make contact with endplates of adjacent fibres (Figures 2 and 3).

Mean endplate surface area did not differ among muscles ($P = 0.065$; Table II). For each muscle, pooled data from all goats suggested a weak positive association between endplate area and fibre cross-sectional area ($r \leq 0.398$; $P = 0.0001$) but this association was not significant in every muscle and did not hold across muscles. Instead, across muscles, there was a weak inverse correlation between endplate area and fibre area ($r = -0.209$; $P = 0.0433$; Figure 4).

The endplate surface area to fibre cross-sectional area (ESA:CSA) ratio was greater ($P < 0.05$) in the laryngeal muscles than in any of the other muscles (Table II). There was a hyperbolic, inverse correlation between the ESA:CSA ratio and fibre cross-sectional area ($r = 0.96$;

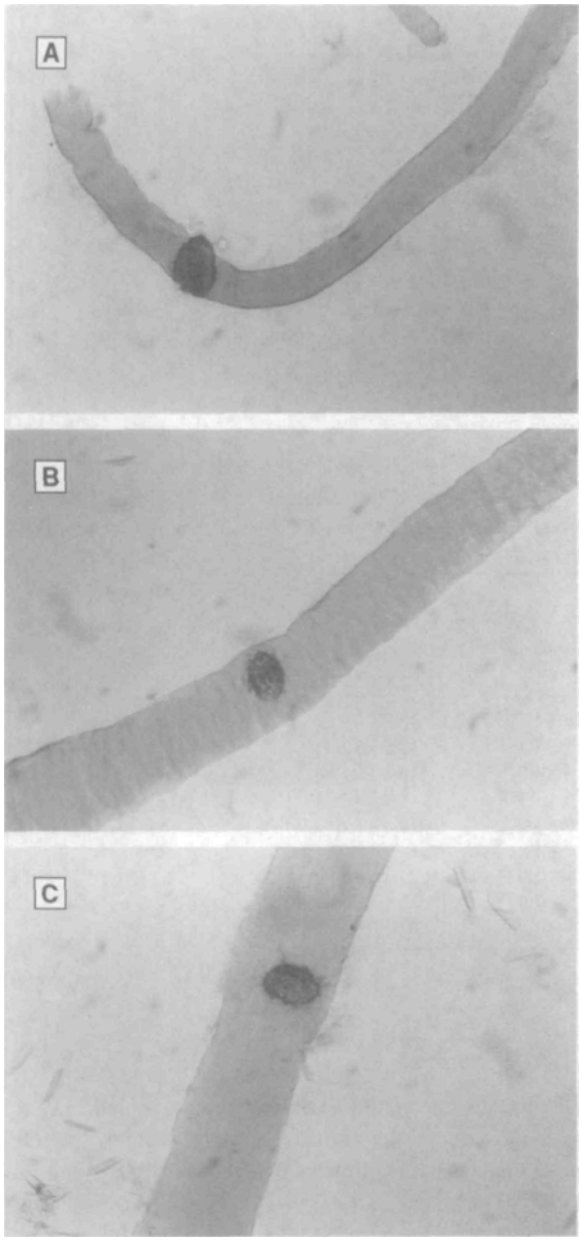


FIGURE 2 Photomicrographs of single muscle fibre segments stained for acetylcholinesterase to outline the motor endplates (dark structures). (A) thyroarytenoideus; (B) gastrocnemius; (C) rectus abdominis muscles (Same magnification). Notice the differences in fibre size, and that the endplate on the laryngeal fibre occupies a larger proportion of fibre surface area than endplates on fibres from the other muscles.

$P = 0.0001$; Figure 5). There was also a weak inverse association between type I fibre composition and the ESA:CSA ratio in muscles ($r = -0.362$; $P = 0.0003$).

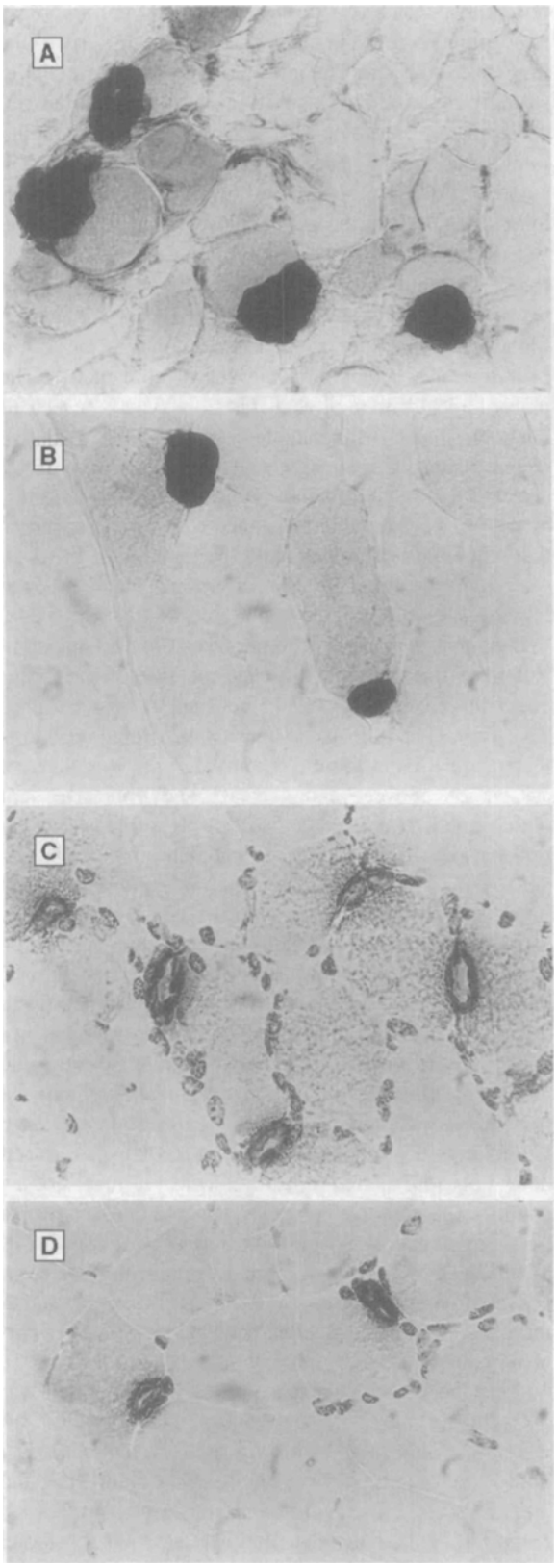
Acetylcholine receptor characteristics

The mean number and density of αBTX binding sites per endplate were least in the masseter ($6.6 \pm 1.3 \times 10^6$ and $8.9 \pm 1.2 \times 10^3$ μm^{-2}, respectively) and largest in the transversus abdominis ($14.5 \pm 1.9 \times 10^6$ and $22.3 \pm 3.5 \times 10^3$ μm^{-2}, respectively). However, neither the number (F-test = 0.084; Scheffe $P > 0.05$) nor the density (F-test = 0.043; Scheffe $P > 0.05$) of αBTX binding sites at the endplates differed among muscles probably because there were marked individual variations (Table II). The number or density of αBTX binding sites at the endplates did not correlate with either fibre type composition, fibre size, endplate size or the ratio of endplate size to fibre size.

Discussion

This study has demonstrated that in the goat (1) fibre size increased and the endplate area to fibre area ratio decreased in the order laryngeal, diaphragm, masseter, limb and abdominal muscles; (2) although there are differences in fibre type composition among these muscles neither fibre composition, endplate size, acetylcholine receptor number nor receptor density differed systematically between the five muscle groups; (3) there is, at best, only a weak positive association between endplate size and fibre size within muscles, and a weak inverse correlation between endplate size and fibre size across muscles.

The eight muscles studied differ in function and response to muscle relaxant drugs, and are of clinical relevance to the anaesthetist. Fibres in these muscles were classified as type I (slow-twitch) or II (fast-twitch) on the basis of their mATPase and oxidative enzyme (SDH or NADH-TR) activities because these enzymes reflect fibre speed of contraction and resistance to fatigue, respectively.[16,17] Fibre cross-sectional area and endplate surface area were measured rather than fibre diameter or the length of motor endplates because they are reported to yield more accurate estimates of fibre and endplate size.[18,19] However, the endplate area estimates obtained did not take into account the ridges and folds of the postjunctional membrane and the curvature of the fibre surface.[12] Similarly, the method used to estimate the number of acetylcholine receptors at the endplates has several potential sources of error: In calculating the number of αBTX binding sites per endplate it was assumed that (1) the gamma counts obtained were due to specific binding since non-specific binding should be negligible compared with specific binding at the endplate because of the short fibre segments used;[20] (2) extrajunctional binding of αBTX was uniform along the length of the fibre although a perijunctional gradient might exist;[21] (3) the thiocholine stain used to identify



the endplates did not affect the number of gamma counts obtained,[12] and (4) each αBTX site corresponded to one acetylcholine receptor. However, it is possible that each receptor binds two αBTX molecules, as it does the neurotransmitter, acetylcholine, in which case the number and density of receptors reported here should be halved. It has been shown that estimates of acetylcholine receptor number obtained by this method are comparable with those obtained by electron microscopic autoradiography.[15]

The fibre type compositions of the eight muscles studied appear consistent with their physiological functions as tonic or phasic muscles. Hence, type I fibres that are resistant to fatigue predominate in the masseter, soleus and diaphragm which are involved in tonic or continuous phasic contractions (rumination, posture maintenance and respiration, respectively), type II fibres with low resistance to fatigue predominate in the gastrocnemius and abdominal muscles which are involved in activities (e.g., jumping and coughing) that require maximum speed for short periods, while type II fibres with high oxidative activity (that is, moderate resistance to fatigue) predominate in laryngeal muscles which are involved in activities (laryngospasm and abduction of the glottis) that require both speed and endurance. Between the thyroarytenoideus and cricoarytenoideus dorsalis muscles, the former contained more type II fibres consistent with its faster contraction speed and lesser resistance to fatigue for laryngospasm compared with glottal abduction, respectively.[22] Similar structural-functional relationships have been reported within and between muscles across mammalian species.[23,24] Consistent with previous reports also in the goat[25–27] is the finding that whereas fibre composition did not differ in a systematic order among the five muscle groups, fibre size increased in the order laryngeal, diaphragm, masseter, limb and abdominal muscles. Although there are no reports on the diameter of fibres in muscles from these five groups in the same subject, and despite difficulties in comparing results from different studies that employed different indices of fibre size, available reports (see Table I in Ibebunjo and Donati[3]) still suggest that in the dog and human fibre size increases in the same order as reported here for the goat.

FIGURE 3 Photomicrographs of transverse cryostat sections stained for acetylcholinesterase activity. (A) thyroarytenoideus; (B) diaphragm; (C) masseter; (D) rectus abdominis muscles (same magnification). With this method, endplates are stained dark while muscle fibres are poorly stained and delineated. Notice that endplates on laryngeal fibres have larger cross-sectional areas than endplates on fibres in the other muscles.

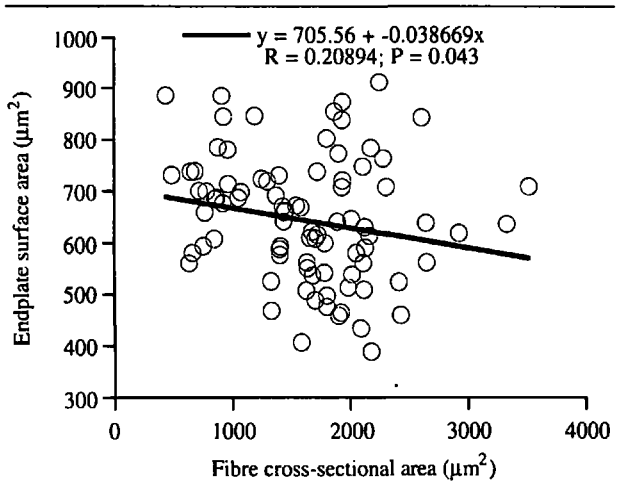


FIGURE 4 Regression analysis illustrating the weak inverse correlation between fibre cross-sectional area and endplate surface area.

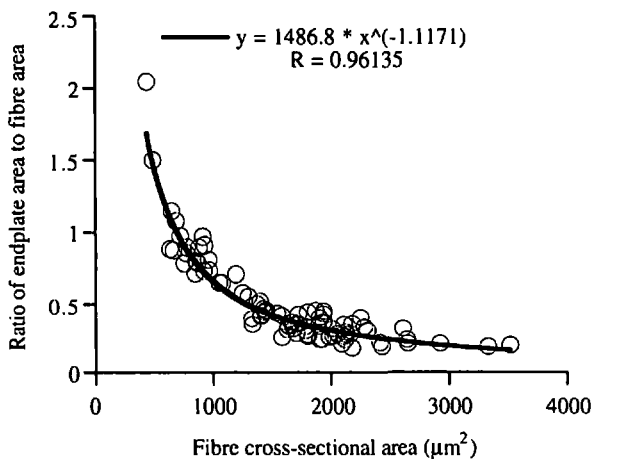


FIGURE 5 The hyperbolic, inverse relationship between fibre cross-sectional area and the ratio of endplate surface area to fibre cross-sectional area.

Fibres in the masseter were histochemically unique in that their mATPase activity at pH 9.4 was not inhibited by preincubation at acidic pH (4.2–4.3). This finding contrasts with a previous report that the goat masseter is composed predominantly of type I fibres which stained weakly for mATPase at pH 10.4–10.6 and strongly at pH 4.3–4.5.[28] This discrepancy might be explained by differences in the alkaline pH of preincubation (9.4 versus 10.4–10.6) between the two studies, or by differences in the levels and duration of masticatory activity between goats depending on the nature of their food (grass and hay or concentrate).[28] Interestingly, individual variations in fibre composition have also been reported in the human masseter, and are suggested to reflect different levels of utilization and varying ability to adapt to jaw-muscle hyperactivity.[29] In muscles of the limb and body trunk, fibres that stain moderately to strongly for mATPase activity at both the alkaline and acidic pH are classified as type IIC. They are commonly found during neonatal development and in certain pathophysiological states, but are rare in normal adult muscles.[11,30] Since the goats studied were healthy, and type IIC fibres were not found in the other muscles, the fibre type in the masseter is unlikely to be type IIC, and was classified as type IM. A similar fibre type has been found in the masseter of other mammalian species but it is not clear whether it is present in humans.[31,32] The physiological importance of this fibre type is not clear, but their high alkaline-stable mATPase and oxidative enzyme capacities suggest that they may have faster speed of contraction (than type I fibres in limb muscles) as well as considerable resistance to fatigue, properties that will appear consistent with the function of the masseter in mastication (a phasic activity) and support of the jaw at rest (a tonic activity).

Despite the marked differences in fibre composition and fibre size between muscles, the size of the motor endplate remained relatively constant across muscles. This finding is surprising in view of previous reports that in the frog,[18] mouse[9,33] and cat[34] there is a direct association between endplate size and fibre size both within and across muscles, as well as a direct association between fibre and hence endplate size versus the amount of acetylcholine released with a presynaptic impulse in the frog.[18] In contrast, we found that the association between endplate size and fibre size within muscles was weak and not significant for every individual muscle,[9] and that fibre size and endplate size were inversely related across muscles. These discrepancies might reflect differences among animal species, the number of muscles studied, or might simply indicate that the association between fibre and endplate size within muscles is coincidental rather than causal and does not hold across muscles. Thus, it may not be assumed that muscles composed of large diameter fibres bear larger endplates and hence release more acetylcholine with a presynaptic impulse than muscles composed of small diameter fibres. It is not clear whether there are differences in mean endplate size between corresponding muscles across mammalian species. In the rat, the mean endplate area in five limb muscles was 500–790 μm², comparable to values in the goat although mean fibre diameter was much smaller in the rat (25–34 μm).[20] Regrettably, other studies[9,18,33,34] investigated other muscles or used a different index (the length of the nerve terminal) to estimate endplate size making direct comparisons of the results difficult.

Similarly, it is not clear whether the mean number or density of αBTX binding sites at the neuromuscular junction differ among muscles or is related to fibre composition, fibre size or endplate size. No differences or relationships were found in this study, probably because of the marked variations among goats. Previous studies based on a comparison of only two muscles yielded conflicting views. In the mouse, ^{125}I-αBTX binding studies showed that receptor number and density per endplate were 34% greater in the soleus than the extensor digitorum longus muscle,[12] but in rats both the number and density of receptors, estimated electrophysiologically, were 60% less in the soleus than the extensor digitorum longus muscles.[35] Another study in the mouse found that the number but not the density of receptors per endplate varied directly with fibre size.[33] The present estimates of the mean number (8.0–14.5 × 10^6) and density (8.9–22.3 × 10^3 μm^{-2}) of receptors per endplate in goat muscles appear less than or comparable to estimates for the rat sternomastoideus (36–47 × 10^6),[36] cat popliteus and extensor digitorum longus (58 ± 6 × 10^6),[34] frog cutaneous pectoris (26,000 μm^{-2} on the thickened postjunctional membrane),[37] or different muscles in the mouse (9.7–35.8 × 10^6 and 8,500–47,800 μm^{-2}).[12,20,33,38,39] It is not clear to what extent differences among species and methods contribute to these discrepancies, or to the marked variations in estimates of receptor number or density between available reports.[12,20,33–39]

In summary, this study has shown that in the goat fibre size increased and the ratio of the endplate area to fibre area decreased in the order laryngeal, diaphragm, masseter, then limb and abdominal muscles, but differences in fibre composition did not follow a similar systematic order between these five muscles groups, and endplate size, receptor number or receptor density did not differ significantly between these muscles. Fibres in the masseter were unique in that they possessed the histochemical properties of both types I and II fibres in limb muscles. It remains to be demonstrated whether similar differences exist in other mammalian species. Nevertheless, these morphological differences might contribute to the unequal responses of muscles to relaxant drugs in so far as muscle response may be related to fibre type composition, fibre size, endplate size and/or acetylcholine receptor distribution in muscles.

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