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Site-dependent difference in the density of sympathetic nerve fibers in muscle-innervating nerves: a histologic study using human cadavers

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Summary

The autonomic nerve supply of skeletal muscle has become a focus of interest because it is closely related to the adaptation of energy metabolism with aging. However, there is no comprehensive information concerning the sympathetic nerves present in muscle-innervating nerves (muscle-nerve). At the point of entry of muscle-nerves into 8 striated muscles (the soleus, extensor carpi radialis, infraspinatus, genioglossus, extra- ocular medial rectus, temporalis, lateral pterygoid, and digastricus anterior belly) in 15 cadavers of elderly people, we counted both tyrosine hydroxylase-positive nerve fibers (TH- fibers) and motor nerve fibers to estimate the ratio of TH-fiber/motor fibers. The 3 limb muscles were found to have a high ratio (soleus, 58%; infraspinatus; 45%; extensor, 36%), whereas the 4 head muscles (digastricus, 23%; genioglossus, 15%; temporalis, 10%; lateral pterygoid, 6%; medial rectus, 1%) had relatively low ratios. The site- dependent characteristics of the TH-fibers seemed to reflect their commitment to muscle activity. However, some discrepant characteristics were noted: 1) In spite of the tonic and continuous activity required for both the genioglossus and infraspinatus, the proportions of TH-fibers were quite different between the tongue and the shoulder muscles; 2) Likewise, the soleus and extra-ocular rectus showed a considerable difference, even though rapid and phasic contraction is essential for both muscles. Rather than reflecting the influence of postnatal functional demand, these site- dependent characteristics might develop as a result of differences in sympathetic innervation of the striated muscles during fetal development, i.e., a short course along the arteries feeding the head muscles, or a long course along the muscle-nerves to the limb muscles.

Key words: Tyrosine hydroxylase-positive nerve fibers – Sympathetic innervation – Striated muscles – Feeding artery – Muscle hilus

Introduction

The autonomic nerve supply of skeletal muscle has become a focus of interest because it is closely related to the adaptation of energy metabolism with aging. Previous research has suggested that autonomic nerve fibers enter a skeletal muscle along feeding arteries, rather than along the muscle-innervating nerve

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(hereafter referred to as “muscle-nerve” for convenience). Therefore, histological studies have concentrated on the nerve elements around and along the peripheral, intramuscular vessels (Fleming et al., 1989; Grasby et al., 1999; Ljung et al., 1999; Guidry and Landis, 2000; Anderson et al., 1996). A limited exception might be found in Li et al. (1995), who demonstrated that sympathetic innervation reached the feeding artery of the gastrocnemius via the sciatic nerve, based on experiments involving sympathectomy. Does the muscle-nerve also contain sympathetic nerve fibers to the muscle?

To examine whether or not a muscle-nerve contains sympathetic fibers, we should pick up the muscle-nerve selectively from the cadaver. A muscle-nerve is known to enter the skeletal muscle at a specific site, the “muscle hilus”, along the muscle surface. The major feeding artery also usually enters the muscle at the hilus, but there are often other, additional arteries entering the muscle distant from the hilus. In the field of reconstructive surgery, such patterns of muscle-nerve and feeding arteries have been described in detail, and classified in 5 types (e.g., Cormack and Lamberty, 1994). For example, the extensor carpi radialis muscle carries a hilus for the muscle-nerve and also other multiple hiluses for the vessels. Ljung et al. (1999) conducted a qualitative examination of tyrosine hydroxylase (TH)-positive nerve fibers (candidate sympathetic nerves; TH-fibers) in the most proximal part of the muscle. However, according to our interpretation they failed to identify the muscle-nerve, although they did examine some of the multiple hiluses for vessels. In addition to difficulty in finding of the hilus, in order to count TH-fibers in the muscle-nerve at the hilus, considerable care is required to avoid “contamination” by sympathetic fibers innervating the skin or viscera. Human skin nerves contain abundant TH-fibers, and hence hand surgeons have performed quantitative or morphometric studies for surgical reconstruction after injury (Auplish and Hall, 1998; Balogh et al., 2002; Chakravarthy Marx et al., 2009, 2010).

Accordingly, using human cadaveric specimens (8 muscles obtained from each of 23 preserved cadavers; for details, see Materials and Methods), the aim of the present study was to examine site-dependent differences in the morphology of sympathetic nerve innervation between muscles. Because of possible variations in the size or amount of nerves, motor units, and muscle fibers themselves, we determined the ratio of TH-fibers relative to thick, myelinated motor nerve fibers at the muscle hilus as a parameter for evaluating these differences.

Materials and Methods

Using 15 cadavers donated for the annual student dissection courses at Tokyo Dental College (6 males, 9 females; mean age, 81.5 years old at death), we investigated muscle-nerves (muscle-innervating nerves; see Introduction) at the hiluses of the muscles studied. This study was approved by the Human Research Ethical Committee of the Dental School. The cause of death in each case had been ischemic heart failure or intracranial bleeding. Experts in gross anatomy among the authors (S.A; G.M.) dissected the muscle hilus of 8 striated muscles: 1) the soleus (a major generator of propulsion force in walking (Williams, 1995); 2) the extensor carpi radialis (a limited muscle reported previously, see Introduction; 3) the infraspinatus (a major generator of rotator cuff tension at the glenohumeral joint (Mochizuki et al., 2008); 4) the genioglossus (the strongest of the extrinsic tongue muscles); 5) the extra-ocular medial rectus, and 3 masticatory muscles (the temporalis, lateral pterygoid and digastricus anterior belly). None of these muscle-nerves are likely to contain sympathetic innervation to glands, skin and/or viscera. However, because of their use for the student dissection course, sites at and around the muscle hilus of the soleus and the 3 masticatory muscles had been exposed to room air, which may have contributed to the poor results of the immunohistochemstry (for details, see Results).

The donated cadavers had been fixed by intravenous injection of non- neutralized 10% (v/v) formalin solution and preserved in 50% ethanol solution for more than 3 months. Post-fixation was not performed after dissection and removal of the muscle-nerve specimens. After routine procedures for paraffin-embedded histology, for each specimen, 7-8 serial cross-sections of the nerve with 5 micrometers thick were prepared. Some sections were stained with hematoxylin and eosin (HE staining) or silver impregnation (Lillie et al., 1980), while others (2-3 per muscle-nerve) were subjected to immunohistochemistry using rabbit polyclonal anti-human tyrosine hydroxylase (1:100 dilution; Chemicon, Temecula, CA). Such a dense solution is adequate for deeply fixed cadavers with a long preservation (Imai et al., 2006; Takenaka et al., 2005). After incubation overnight at 4°C, the sections were washed. The second antibody (Dako Chem Mate Envison Kit, Dako, Glostrup, Denmark) was labeled with horseradish peroxidase (HRP), and antigen-antibody reactions were detected using the HRP-catalyzed reaction with diaminobenzidine (with hematoxylin counterstaining). The sections were then washed again and mounted in resin solution in xylene after dehydration. All staining images were compared with those of the negative controls, and the expression of protein was confirmed. In contrast to the present paraffin-embedded histology, previous reports of human TH-fibers were based on cryostat sections of cadaveric specimens (Tajti et al., 1999; Thakker et al., 2008; Ibanez et al., 2010).

After practice at identifying thick myelinated fibers in silver-impregnated sections (Fig. 1), the first author (T.N.) counted 1) TH-fibers and 2) thick myelinated fibers (over 10 micrometers in the maximum diameter) under a x40 objective lens. We considered the latter to be the most likely candidates for the axons of alpha motor neurons, but we were not able to exclude thick sensory fibers for the muscle. We used an ocular micrometer set in front of the eye. For each of the muscles examined, the counting was performed for 3 nerves (i.e., nerve fascicles) cut transversely (or different sections along the nerve when the targets were 1 or 2 in number) at 1 muscle hilus, and we used the middle value for statistical analysis (Student's t test). When we found many nerves containing TH-fibers, we chose the 3 nerves that exhibited the best staining conditions.

Among commercially available antibodies against various autonomic nerve markers such as neuronal nitric oxide synthase (nNOS) and choline acetyltransferase (ChAT), only TH is applicable for "nerve fibers" (not ganglion cells) obtained from donated cadavers after prolonged preservation in 50% ethanol solution (Takenaka et al., 2005; Imai et al., 2006). However, in Japan deep fixation and long preservation are still routine in most medical and dental schools for economic and other reasons.

Results

Overview of the results of immunohistochemistry

The quality of the immunostaining appeared to depend on the conditions under which each cadaver had been fixed and preserved, as well as the site of muscle. Among the specimens from 15 cadavers, we chose the following sections for counting of nerve fibers: 5 specimens for the soleus; 10 for the extensor carpi radialis; 9 for the infraspinatus; 10 for the genioglossus; 3 for the digastricus anterior belly; 3 for the temporalis; 3 for the lateral pterygoid; 8 for the extra-ocular medial rectus. Thus, in more than half of the cadavers, the head muscles exhibited no or only a very weak immunoreaction, possibly because of exposure of the materials to air before dissection (see Materials and Methods). However, in spite of the difficult conditions, the results were sufficient for the demonstration of qualitative and quantitative differences (see below). We counted the nerve fibers of 3 muscle-nerves for each of muscles examined and used the middle value of the three for comparison of proportions.

However, nerve fibers per muscle-nerve were usually quite different in number between specimens (more than 100%): a thick nerve bundle was cut transversely in one specimen, whereas a thin bundle in another specimen. Thus, the standard deviation is shown for the proportion of TH-fibers (Table 1).

Routes of TH-fibers into muscle: via muscle-nerves or along feeding arteries (Figs. 1 and 2)

In 6 muscles other than the extensor carpi radialis and the digastricus anterior belly, the muscle-nerve entered the muscle with the feeding artery. Conversely, in the extensor and digastricus, the muscle-nerve did not accompany any artery or vein at the hilus. Figure 1A shows a muscle hilus of the infraspinatus. TH-fibers running along the artery were often difficult to count because a plexus had formed around the artery or the TH-fibers followed a highly tortuous course. However, in each of the cadavers examined, TH-fibers running

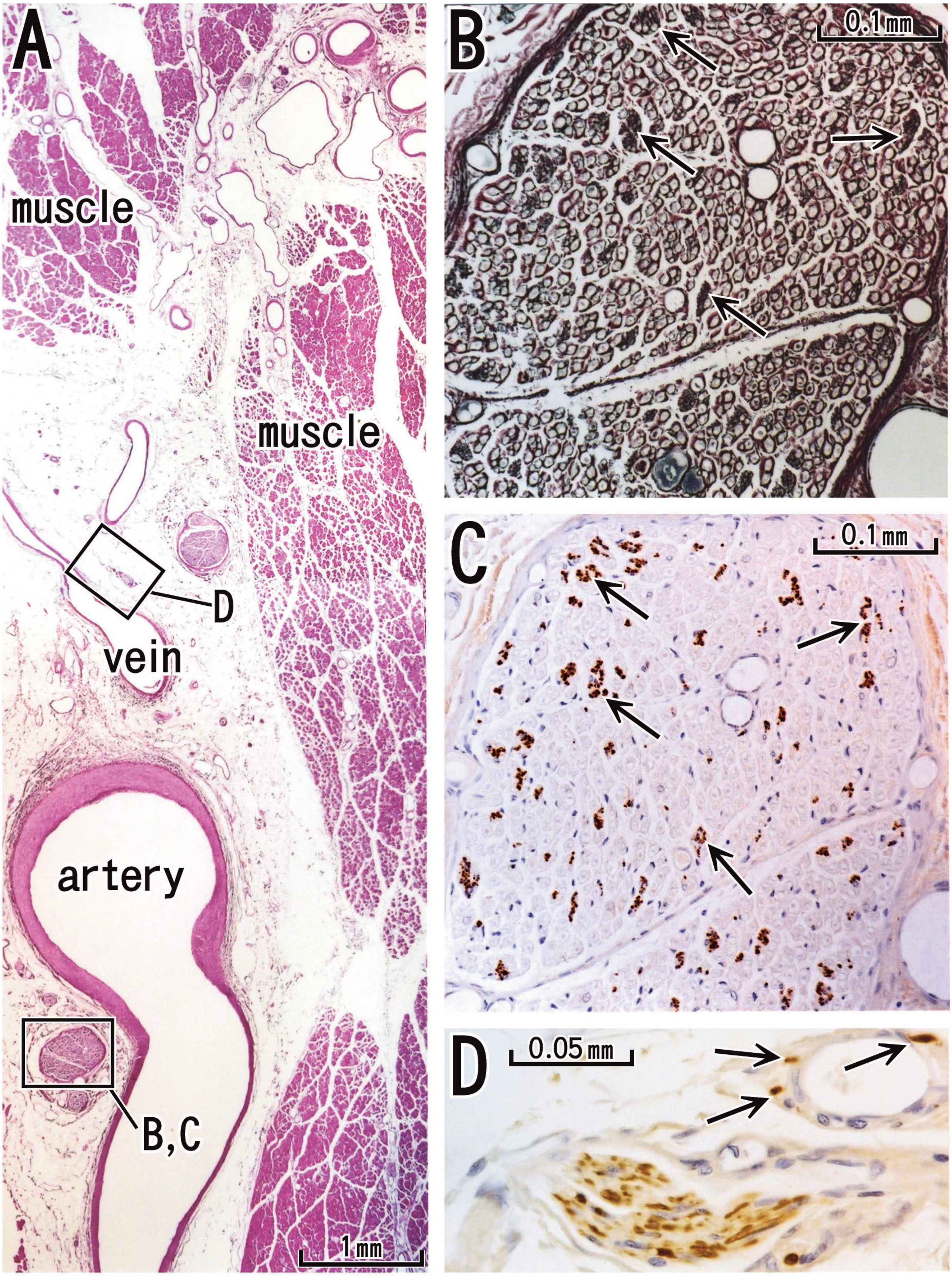

Fig. 1. Muscle hilus of the infraspinatus muscle of a 60-year-old man.
Panel A is a lower-power view of the muscle hilus (HE staining). Panels B-D are higher magnification views of squares in panel A. Panel B (silver impregnation) shows a muscle-nerve containing abundant thick myelinated fibers (similar to open circles), i.e., candidate motor fibers. In panel B, candidate sympathetic fiber bundles are identified as black structures (arrows). Panel C (or Panel D) displays tyrosine hydroxylase immunohistochemistry of a muscle nerve (or the thin nerve along the artery). Arrows in panel C correspond to those in panel B. Panel D also contains tyrosine hydroxylase-positive fibers around an arteriole (arrows).

Table 1. Site-dependent difference in TH-fiber proportions.

	cadavers examined	TH-fibers	motor fibers	proportion-1	proportion-2
Soleus	5	81	140	58±19%	48±16%
Infraspinatus	9	89	197	45±12%	
Extensor carpi	10	38	107	36±16%	
Genioglossus	10	43	295	15±10%	15±14%*
Digastricus anterior	3	10	44	23±13%	
Temporalis	3	17	171	10±8%	
Lateral pterygoid	3	8	139	6±2%	
Extra-ocular rectus	8	2	234	1±1%	

proportion-1 (Mean±SD) per a single muscle-nerve: TH-fibers/motor fiber candidates
proportion-2 (Mean±SD): limb muscles total, head muscles total
* Significant difference between limb muscle(soleus, infraspinatus, extensor carpi) & head muscle(genioglossus, digastricus, temporalis, pterygoid, extra-ocular rectus) at $p<0.01$ by t-test

along the feeding artery were consistently most evident in and around the genioglossus, where abundant thin nerves 0.05-0.1 mm thick contained TH-fibers and were accompanied by arteries (Fig. 2). Although the TH-fibers were far fewer, other head muscles also received such nerves along the arteries. In the extra-ocular medial rectus in particular, few TH-fibers ran along the artery (Fig. 2D), despite the presence of almost the same number of candidate motor fibers per nerve as that in the genioglossus and medial rectus, i.e., a

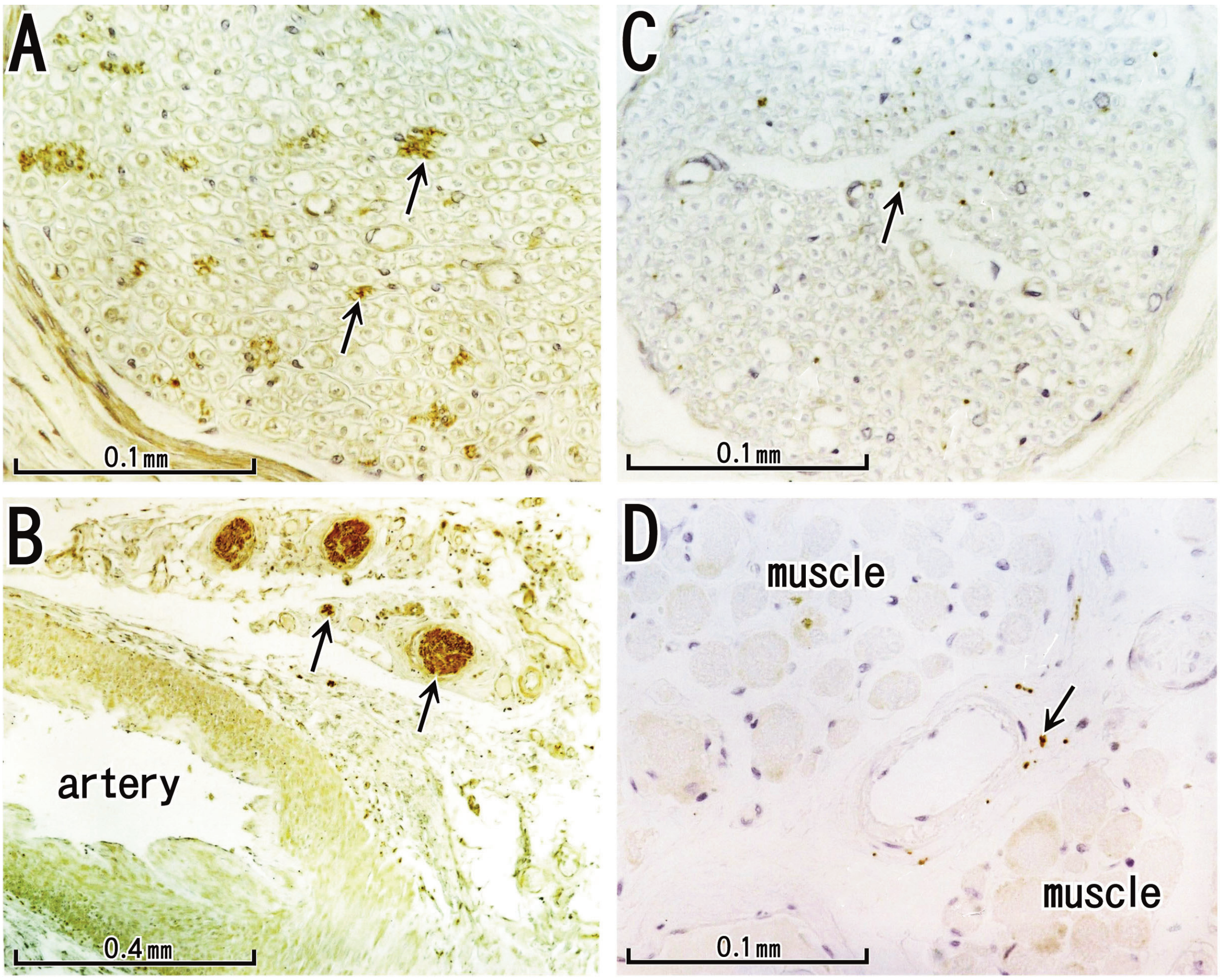

Fig. 2. Tyrosine hydroxylase-positive fibers in the tongue and extra-ocular muscles.
Panel A (or Panel B) shows a muscle- nerve (or an artery and accompanying nerves) in the genioglossus of the tongue (an 87-year-old man). Panel C (or Panel D) exhibits a muscle-nerve (or an arteriole and accompanying nerve fibers) at the muscle hilus of the extra-ocular medial rectus (an 84-year-old man). Panel C displays a site in which the highest density of candidate sympathetic nerve fibers in the specimens examined can be seen. In all panels, arrows indicate tyrosine hydroxylase-positve fibers.

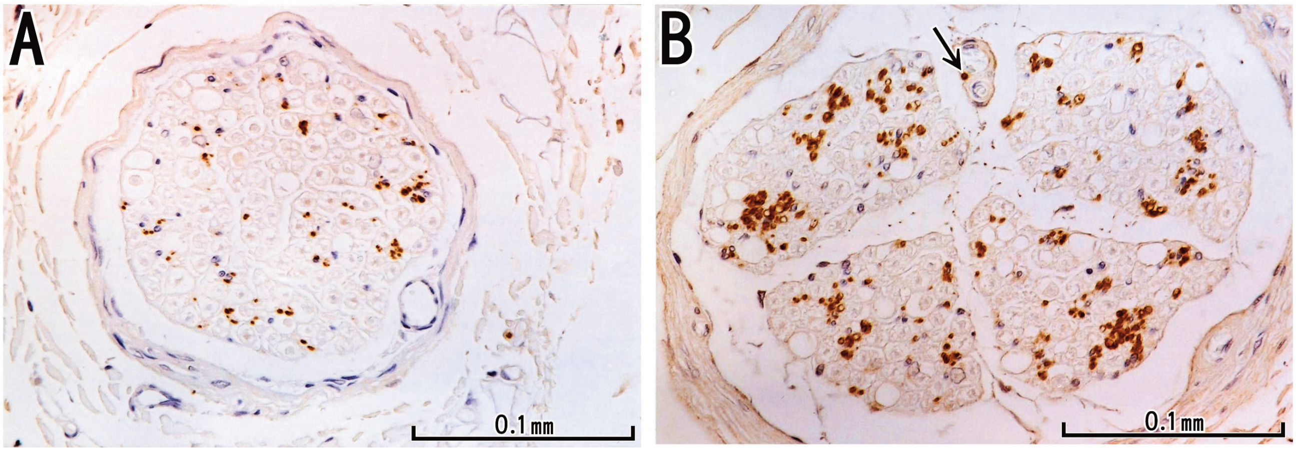

Fig. 3. Abundant tyrosine hydroxylase-positive fibers in muscle-nerves to the extensor carpi and soleus muscles.
Panel A displays a muscle-nerve to the extensor carpi radialis (a 65-year-old man), while panel B the soleus (a 79-year-old woman). Panel B also contains a tyrosine hydroxylase-positive fiber around an arteriole (arrow).

mean of 295 vs. 234 (Table 1). At the hilus of the limb muscles examined, nerves for TH-fibers were also seen running along and near the arteries and veins, but they appeared to contain lower amounts of TH-fibers than those in the muscle-nerves (Figs. 1 and 2). Overall, the amount of "TH-fibers along the feeding artery" appeared to decrease in the following order: genioglossus > head muscles other than the medial rectus > limb muscles > medial rectus.

Site-dependent differences in the proportion of TH-fibers per thick myelinated nerve fiber at the muscle hilus (Figs. 1-3; Table 1)

Although the absolute numbers of nerve fibers (TH-fibers and candidate motor fibers) varied considerably between nerves or between cadavers, the proportion of TH-fibers exhibited a clear site-dependent difference. The thick myelinated fibers that we counted should have contained sensory fibers for muscles, but we considered that the TH-fiber proportion was available for comparison between muscles (Table 1). The 3 limb muscles represented a high-proportion group (soleus, 58%; infraspinatus; 45%; extensor, 36%), in contrast to the 4 head muscles (digastricus, 23%; genioglossus,15%; temporalis, 10%; lateral pterygoid, 6%; medial rectus, 1%), which showed relatively low proportions. In nerves supplying the masticatory muscles, it was difficult to interpret the data because of the low number of specimens. In contrast to nerves running along and near the feeding artery (Figs. 1D and 2B,D), TH-fibers were distributed almost evenly in muscle-nerves at the hilus (Figs. 1C, 2A,C and 3A,B). However, in cross-section, they tended to be distributed more frequently at the periphery than in the center of the nerve. The nerves supplying the limb muscles were each composed of 2-8 nerve fascicles, depending on the nerve thickness, but almost all nerve fascicles contained TH-fibers. TH-fibers formed 1 or 2 clusters in a thick fascicle. Although the muscle-nerve often contained relatively thick vessels between the fascicles, TH-fibers were not concentrated around the "intra-nerve" vessels (e.g., Fig. 3B).

Taken together with the data for TH-fibers running along the feeding artery (see subsection above), TH-fibers in the muscle-nerve appeared to be more numerous than those running along the arteries in limb muscles, whereas the opposite situation was evident in all of the head muscles examined.

Evaluation of differences in immunoreactivity among specimens

We considered it important to compare the results for multiple muscles obtained from a single cadaver because the immunoreactivity was likely to differ depending on the postmortem conditions, such as the time-lag before fixation, rather than on the sites of the muscles. Moreover, it has been reported that nerve-related enzymes are considerably reduced in both amount and activity in patients with diabetic mellitus or those receiving anti-cancer drugs (Taguchi et al., 1999), whereas peripheral nerve TH activity is upregulated in patients with hypertension (Cabassi et al., 2002). Therefore, we examined the data for the soleus, extensor carpi, infraspinosus and genioglossus, because measurements were possible for at least 3 of these muscles in the following 4 cadavers.

In specimen Nº. 1923 (a 94-year-old woman), the proportion of TH-fibers was higher than the mean in all 4 muscles, whereas in specimens Nº. 1922 and 1955 (women aged 90 and 83 years, respectively) the proportion was lowest in 3 of the 4 muscles. However, in the latter 2 cadavers the muscles showing the highest value differed. In another cadaver (No. 1921; a 78-year-old woman), lower and higher values were evenly distributed: 2 of the former and 2 of the latter. Taken together, the results suggested that the proportion of TH-fibers tended to be high or low depending on the cadaver examined, whereas the muscle showing the highest value did not appear to vary very much among the cadavers.

Statistical analysis of site-dependent differences in proportion of TH-fibers

The proportion of TH-fibers supplying the extra-ocular medial rectus displayed significant differences from all the other muscles examined ($p<0.01$); among the head muscles, the medial rectus carried markedly few TH-fibers along the muscle- nerve. The next-lowest proportion was seen in the tongue genioglossus, which exhibited a significant difference from the infraspinatus, extensor carpi, soleus and lateral pterygoid muscles ($p<0.01$). Conversely, the extensor carpi and soleus (i.e., high ratio) showed significant differences from any of the head muscles examined ($p<0.01$). However, no significant differences were evident among the 3 limb muscles examined because of large interindividual differences between nerves or between cadavers in the proportion of TH-fibers in the infraspinatus (max-min, 120%-25%). Also, because of the relatively small numbers of head muscle specimens examined the data for the infraspinatus did not seem to differ markedly from those of the lateral pterygoid, digastricus or temporalis ($p<0.5$). Nevertheless, we were able to conclude that limb muscles carried greater proportions of TH-fibers than head muscles ($p<0.01$; Table 1).

Discussion

Although the number of specimens examined was limited, the present study demonstrated that 1) being independent of the feeding artery, abundant sympathetic nerve fibers enter a striated muscle along the muscle-nerve especially in limb muscles; 2) the proportion of sympathetic nerve fibers per motor nerve candidate in the muscle-nerve displayed a significant site-dependent variation, being high in limb muscles and low in head muscles. These two findings seem to be closely related, because the proportion might be small when most of the sympathetic nerve fibers enter the muscle along the feeding artery. However, in spite of the large number of motor nerve fibers supplying the extra-ocular medial rectus (which is well known to have a small motor unit), few TH-fibers were found running along both the muscle- nerve and artery. Moreover, at the muscle hilus, the feeding artery for each of the soleus and infraspinatus muscles was accompanied by an appreciable number of TH- fibers, in spite of the high proportion of TH-fibers in the muscle-nerve. Therefore, the actual difference in the amount of sympathetic nerves was most likely attributable to inter-muscle variation. The present methodology included several limitations such as a possible contamination of thick sensory fibers for the muscle (not for the skin) and a large difference in the numbers of fibers at a cross section between specimens.

Nevertheless, we considered that the site-dependent difference in the proportion of TH-fibers merits further discussion. In fact, statistical significance was found between the proportions (see the final paragraph in the Results). Does the site-dependent difference in TH-fiber proportion depend on the muscle fiber type? The TH-fiber proportion was highest in the muscle-nerve to the soleus in the muscles examined. The soleus is the strongest muscle generating force for propulsion in walking, and in humans it is well known to have a high content of slow, fatigue-resistant, type I muscle fibers (Williams, 1995). According to Soukup et al. (2002), the rat soleus also contains 96.1% type I fibers. In experimental animals, the soleus has often been compared with the extensor digitorum longus, because the latter is known to have a high content of type 2B fast muscle fibers (75.7%, according to Soukup et al., 2002). The extensor carpi radialis, in which the second highest density of TH-fibers was found, is likely to show a fiber pattern similar to that of the extensor digitorum. Thus, fiber types 1 and 2B are likely to be connected with high proportion of TH-fibers along the muscle-nerve.

In contrast, the extra-ocular rectus showed the lowest density of TH-fibers. In rats, the extra-ocular muscle contains a specific myosin heavy-chain isoform: the EO type (Rubinstein and Hoh, 2000; Kabanna and Porter, 2004). The medial and lateral rectus muscles are divided into a major global layer and a minor orbital layer, especially during fetal development: the latter contains multiple rare isoforms quite different from other skeletal muscles (Rubinstein et al., 2004). Therefore, the significant difference between the soleus and extra-ocular medial rectus may be explained in terms of the notion that type 1 fibers probably require much denser sympathetic innervation than extra-ocular-type fibers. In addition, the rat temporalis shows a typical regional difference, types 2A- and 2X being dominant in the deep anterior temporalis, and type 2B being dominant in the superficial anterior temporalis and posterior temporalis (Tanaka et al., 2008). Hitherto, however, there has been little or no information on human muscle fiber types, especially on the basis of comparative studies.

Rather than focusing on muscle fiber types, we will discuss the site- dependent differences in TH-fibers in relation to muscle function and/or the capacity for adaptation against fatigue. In the extremities, a distally located muscle generally acts specifically during a single phase of movement (e.g., the soleus, and the extensor carpi), whereas a proximally located muscle (e.g., the infraspinatus) acts to stabilize the joint for distal muscle action during all phases of movement. However, the fastest contraction seems to be required in the extra-ocular muscles. Maintenance of tension or tonus is also necessary for tongue muscles (e.g., the genioglossus), because they function to avoid obstruction of the airway, even during sleep. In addition, the muscles of the lower extremity need to bear the weight of the body during different types of activity, in contrast to the upper extremity muscles. However, in light of these considerations, our findings were discrepant as regards certain issues: 1) In spite of the tonic and continuous activity required for both the genioglossus and infraspinatus, their proportions of TH- fibers were quite different; 2) Likewise, a considerable difference was also evident between the soleus and extra-ocular rectus, even though both must show rapid and phasic contraction. Therefore, rather than functional demands, the site-dependent characteristics of sympathetic innervation to muscles are likely attributable to other reasons, such as factors during fetal development.

In human adults, there are multiple or various sympathetic pathways to a single target, such as the ciliary muscle of the iris, including independent dedicated nerves for TH-fibers, TH-fibers running along the abducent and trochlear nerves, and TH-fibers running along the arteries (Barjet et al., 1989; Oikawa et al., 2004; Thakker et al., 2008). Moreover, 4 peripherally located autonomic ganglia (ciliary, pterygopalatine, otic and submandibular) contain varying proportions of TH-positive neurons in addition to the major parasympathetic neurons in humans, as well as in experimental animals (Uemura et al., 1987; Leblanc and Landis, 1989; Hardebo et al., 1992; Kirch et al., 1995; Tan et al., 1995; Ng et al., 1995; May et al., 2004; Rusu and Pop, 2010). Previous studies have demonstrated higher numbers of TH-positive neurons in fetuses than in adults. Therefore, in the head region, a mixed nerve trunk, such as the sciatic or radial nerve in the limb, seems to be rare; possibly only the mandibular nerve corresponds to this category in the head. During fetal development, when neural crest cells migrate along short and multiple courses (O'Rahilly and Müller, 2007), the commitment of multiple or various sympathetic pathways to a single target would probably occur. Because TH-fibers for the head muscles are likely to take a course independent of the muscle-nerve, the head muscles might show a low density of TH- fibers at the muscle-hilus. In the present study, the amount of "TH-fibers along the feeding artery" appeared to be highest in the tongue muscles. In fact, the fetal tongue root is located in the immediately anterior side of the superior cervical ganglion (Rodríguez-Vázquez et al., 2011). Thus, the TH-fibers seem to easily chose a short course along the feeding artery running along the antero-posterior axis. In mid-term fetuses, in contrast to other head muscles, we observed abundant TH-fibers running into the tongue (unpublished data). Conversely, a long course of sympathetic nerves to limb muscles is likely to facilitate the fasciculation or involvement into the muscle-nerve. Nevertheless, these anatomical considerations seem unable to explain why the amount of TH-fibers was extremely low in the extra-ocular muscle, not only along the muscle-nerve but also along the feeding artery.

The present study focused TH fibers along the muscle-nerve, but the core of the results seems to be consistent with previous knowledge, i.e., limb muscles carry greater numbers of TH-fibers than head muscles. To our knowledge, the present study is the first to describe the perspective of the sympathetic components of the "muscle- nerve" at the muscle hilus. Our trial seems to contribute to a quantitative evaluation of the sympathetic nerve supply to striated muscles. However, in the peripheral course in the muscle, the "muscle-nerve" is most likely to give off TH-fiber components to the smooth muscle coat of the arteriole. Thus, we observed a sympathetic nerve at a site in the small central or proximal side of the previous research interests (i.e., the most peripheral site). Nevertheless, at the muscle-hilus there were 2 types of sympathetic nerve morphologies: 1) thin tortuous nerves, containing only TH-fibers and 2) TH-fibers mixed with motor fibers in the muscle-nerve. We speculate the existence of a difference in targets between these 2 types of TH-fibers. Do TH-fibers in the muscle nerve carry specific targets other than the vascular smooth muscle coat? Indeed, TH-fibers are likely to innervate 1) the intrafusal muscle fibers of muscle spindles (Bombardi et al., 2006), 2) pericytes in the muscle (Fukutani et al., 2009) and 3) muscle enthesis i.e., the tendon and other muscle-associated connective tissues at the origin and insertion (Ljung et al., 1999; Danielson et al., 2007). Further study is necessary to follow the TH-fibers from the muscle hilus to the possible multiple targets.

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