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COMPARISON OF SKELETO-FUSIMOTOR INNERVATION IN CAT PERONEUS BREVIS AND PERONEUS TERTIUS MUSCLES

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SUMMARY

1. The skeleto-fusimotor or β innervation was compared in cat peroneus brevis and peroneus tertius muscles, which differ in their composition of fatigue-resistant motor units; the slow (S) units predominate in brevis and the fast units (FR) in tertius.

2. In four brevis muscles, of thirty-four β-axons (from a total of 114 axons supplying extrafusal muscle fibres) twenty-nine were dynamic (βD) and only five static (βS). In contrast, in three tertius muscles, of twenty-five β-axons (from a total of 82 axons) twelve were static and thirteen dynamic.

3. In a population of thirty-five brevis and thirty tertius spindles, the proportion of βD-innervated spindles was greater in the brevis (68·5%) than in the tertius (50%) whereas that of βS-innervated spindles was greater in the tertius (40%) than in the brevis (17·1%). In a population of thirty-two brevis and twenty-seven tertius spindles in which the presence of bag_1 fibres was deduced from the existence of a dynamic innervation, the proportion of spindles innervated by βD-axons was 80% in the brevis and 62% in the tertius.

4. In both muscles, the number of βD effects was greater than that of βS effects. βS-axons were rarely found to supply more than one spindle whereas βD-axons supplying more than one spindle (up to four) were common. Spindles were often co-innervated by βD- and βS-axons.

5. In brevis as well as in tertius, spindle effects elicited by βD-axons were nearly as frequent as effects by γD-axons whereas static effects elicited by γS-axons were much more numerous than βS effects.

INTRODUCTION

The peroneus tertius (PT) and the peroneus brevis (PB) muscles differ in the proportion of slow (S) and fast fatigue-resistant (FR) motor units. In peroneus brevis, S units predominate (44% S compared to 24% FR), whereas a reverse proportion is observed in peroneus tertius (23% S and 42% FR) (Emonet-Dénand, Hunt, Petit & Pollin, 1988). The fact that dynamic skeleto-fusimotor axons (or βD-axons) have been shown to supply S units (Barker, Emonet-Dénand, Harker, Jami & Laporte, 1977; Jami, Murthy & Petit, 1982) and static β-axons FR and occasionally FF (fast fatigable) units (Jami *et al.* 1982) led us to compare the β

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innervation in these two muscles with the aim of verifying the relation of βD-axons with slow units and that of βS-axons with fast units.

This study was also prompted by the difference in the proportion of dynamic β-innervated spindles reported in brevis, 75%, by Emonet-Dénand & Laporte (1975) and in tertius, less than 40%, by Jami *et al.* (1982). Furthermore, there has not been a systematic study of static β innervation in peroneus brevis.

In each experimental muscle, the aim was to study the effect of the largest possible number of single motor axons of all conduction velocities on each of the several single Ia afferent fibres which had been prepared.

METHODS

The experiments were carried out on adult cats (2–3·5 kg) anaesthetized with pentobarbitone sodium (Nembutal, Abbott Laboratories, 40 mg/kg I.P.). A constant level of anaesthesia was maintained for the duration of the experiment by regular intravenous injections of small amounts of diluted Nembutal (1 or 2 ml of a solution of 6 mg/ml). The temperature of the animal and that of the experimental muscle was kept at 37 °C by a pad and by a thermoregulated resistor immersed in the paraffin pool protecting the muscles.

A laminectomy was performed for exposure of the lumbar cord. The hindlimb was extensively denervated except for the peroneus tertius or for a part of the peroneus brevis. In the latter, only one nerve branch to the muscle was kept intact so as to facilitate the search for β-axons (see Emonet-Dénand & Laporte, 1975). The muscle tendon was directly attached to a myograph (Kulite load cell BG 1000 with a compliance of 1·3 μm/100 g) rigidly mounted on a servo-assisted electromagnetic puller. The nerve to the experimental muscle was dissected free for over 10 mm near its entrance into the muscle and held in paraffin oil on a single recording electrode, the other electrode being in contact with a neighbouring muscle. Single afferent and single motor axons were prepared by splitting dorsal and ventral root filaments (S1, L7 and L6 when necessary). Each axon was considered as functionally single when its threshold stimulation in a filament elicited an all-or-none potential in the muscle nerve which was not followed by another potential for a ten times threshold stimulation. The conduction velocity of each axon between the root filament and the recording electrode was calculated from the latency between stimulus artefact and the action potential.

Afferent fibres were first selected for their high sensitivity to small amplitude (0·5–1 mm) sinusoidal (1 Hz) muscle stretch. They were later identified as Group Ia fibres supplying spindle primary endings if their conduction velocity was higher than 70 m/s and if their discharge was increased by repetitive stimulation of a γ axon. On average, the tertius contains fifteen spindles, the brevis forty (Scott & Young, 1987). In each experiment from seven to twelve single Ia afferent fibres were prepared (see Table 1); in tertius, on whole muscles but in brevis, on only a part of the muscles, for the above mentioned reason. This was possible because the brevis is supplied by two nerve branches (rarely three), a rostral and a distal, the latter being usually thicker. In three experiments on brevis (PB 1, 2 and 3 (see Table 1)) only the rostral branch was kept intact; in PB 4, only the distal. Each filament with a single Ia afferent fibre was mounted on a branch of a multiple recording electrode, so as to readily select the discharge of any of the Ia fibres.

Single motor axons whose stimulation in ventral root filaments elicited the contraction of a motor unit were prepared. In the three experiments on tertius (PT, 1, 2 and 3) twenty-six to twenty-nine of such axons were prepared; in three experiments on brevis (PB, 1, 2 and 3) from nineteen to twenty-five axons and in PB 4, thirty-nine axons were prepared. Since, on average, the tertius is supplied by thirty-four α-axons and the whole brevis by seventy-five α-axons (Horcholle-Bossavit, Jami, Thiesson & Zytnicki, 1988), it means that a very large proportion of these axons was studied in each experimental muscle. Then, by using various techniques to completely eliminate extrafusal contractions, some of these axons were identified as β if the spindle activation they elicited persisted in this condition. For βD-axons the selective blockade of extrafusal junctions may be readily obtained during prolonged stimulation at relatively high frequency (Emonet-Dénand & Laporte, 1974) whereas for βS, repeated short periods of stimulation at relatively low frequency are necessary in cats (Jami *et al.* 1982) which is not the case in rabbits (Emonet-Dénand, Jankowska & Laporte, 1970). Further classification of the axons as dynamic or static rested on the effect of these axons on the responses of primary endings to ramp-and-hold stretch.

The type of motor units (S, FR, FF, FI (fast intermediate) of Burke, Levine, Tsairis & Zajac's classification, 1973) supplied by β-axons was identified with the protocol used by Petit, Filippi, Emonet-Dénand, Hunt & Laporte (1990).

While searching for β-axons, as many as possible fusimotor (γ) axons (identified by the longer latencies of their action potentials in muscle nerves following ventral root stimulation and by the lack of muscle action potentials) were prepared and the effect of each of them was tested on the discharge of each primary ending. Whenever a γ-axon activated a primary ending, its action – static or dynamic – was determined. Sometimes an axon exerted an unclassifiable effect on a primary ending (category III of Emonet-Dénand, Laporte, Matthews & Petit's classification, 1977) but the axon could be classified by the effect it had on other spindle(s).

Stimulation sequences and data collection were carried out using an Epson XT computer with a Tecmar Labmaster card.

RESULTS

The comparison of β innervation in peroneus tertius and peroneus brevis muscles (proportion of βD and βS, percentage of β-innervated spindles) is followed by a quantitative comparison of spindle γ and β supplies.

β-axons

The proportion of β-axons among those supplying extrafusal muscle fibres was the same in the two muscles: 29·8% in the brevis (34 β-axons out of 114 axons) and 30·4% in the tertius (25 β-axons out of 82 axons) (see Table 1). However, the repartition of βS- and βD-axons was very different in the two muscles: βD-axons largely predominated in the brevis (of thirty-four β-axons, twenty-nine were dynamic and five static) whereas in the tertius, βS were nearly as common as βD (of twenty-five β-axons, twelve were static and thirteen dynamic).

The conduction velocities of βS-axons were comparable in brevis and tertius: they were all in the 75–95 m/s range (Fig. 1). That was not the case for βD-axons: in tertius no βD-axon faster than 75 m/s was observed (range: 55–75 m/s) whereas in brevis some βD-axons had conduction velocities faster than 75 m/s (range: 50–85 m/s).

Percentage of β-innervated spindles

This percentage was high in both muscles: 74·2% in the brevis (twenty-six β-innervated spindles out of thirty-five) and 67·7% in the tertius (twenty-one β-innervated spindles out of thirty). The βS-innervated spindles were more frequent in tertius than in brevis. In tertius, twelve spindles out of thirty were βS innervated (40%), six by βS alone and six in common with βD, whereas in brevis only six spindles out of thirty-five were βS innervated (17·1%), two by βS alone and four in common with βD.

The βD innervation predominated in both muscles. In brevis twenty-four out of thirty-five spindles (68·5%) were βD innervated, twenty by βD alone and four in common with βS. In tertius, fifteen out of thirty spindles (50%) were supplied by βD-axons, nine by βD alone and six in common with βS.

In the peroneus muscles, 10–20% of the spindles lack a bag_1 fibre (Scott & Young, 1987). When the percentage of βD-innervated spindles was calculated for only those

TABLE 1. Comparison of the skeleto-fusimotor supply in cat brevis (PB) and tertius (PT) peroneus muscles.

				β dynamic							β static						
							No. of β-axons							No. of β-axons			
Muscles	n Ia	n ax.	n β	N	m.u.	1 sp.	2 sp.	3 sp.	4 sp.	Sp. eff.	N	m.u.	1 sp.	2 sp.	3 sp.	4 sp.	Sp. eff.
Peroneus brevis																	
PB 1	9	19	6	4	S	2	1	—	1	8	2	FR	2	—	—	—	2
PB 2	11	31	16	14	S	8	4	2	—	22	2	FR	2	—	—	—	2
PB 3	7	25	4	3	S	3	—	—	—	3	1	FR	—	1	—	—	2
PB 4	8	39	8	8	S	5	1	2	—	13	—	—	—	—	—	—	—
Peroneus tertius																	
PT 1	10	26	12	6	S	1	3	1	1	14	6	FR	5	1	—	—	7
PT 2	8	27	6	4	S	3	1	—	—	5	2	FR	2	—	—	—	2
PT 3	12	29	7	3	S	1	1	—	1	7	4	1FR	1	—	—	—	1
												3FF	3	—	—	—	3
Total																	
4 PB	35	114	34	29		18	6	4	1	46	5		4	1	—	—	6
3 PT	30	82	25	13		5	5	1	2	26	12		11	1	—	—	13

n Ia, number of spindle Ia fibres; n ax., number of motor axons supplying extrafusal muscle fibres; n β, number of skeleto-fusimotor (β) axons.

N, number of β axons; m.u., type of motor units supplied by these axons; note that all βD-axons supplied slow (S) units whereas fourteen βS-axons supplied fast-fatigue-resistant (FR) units and three fast-fatigable (FF) units.

No. of β-axons; the number of axons supplying 1, 2, 3 or 4 spindles respectively; note that nearly all βS-axons supplied only one spindle whereas many βD-axons supplied several spindles.

Sp. eff., total number of spindle effects elicited by β-axons.

In PB 1–3 experiments, the nerve supply to the muscles was limited to the rostral branch, in PB 4 experiment, to the distal branch. The composition in motor units of the part of the muscle supplied by this branch was similar to that described by Emonet-Dénand *et al.* (1988) for the part of the brevis supplied by the rostral branch: of the thirty-nine motor axons supplying extrafusal muscle fibres studied in PB 4 experiment, seventeen were S units, ten FR, eleven FF and one FI.

spindles supplied by dynamic axons, which indicated the presence of a bag_1 fibre (thirty out of thirty-five brevis spindles and twenty-five out of thirty tertius spindles), the percentage of βD-innervated spindles was 80 % in the brevis and 62 % in the tertius.

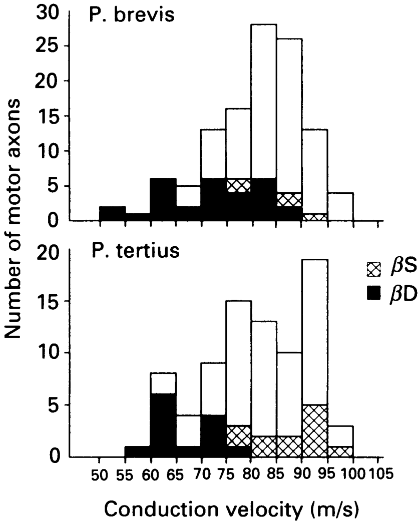

Fig. 1. Histograms of the conduction velocity of motor axons supplying extrafusal muscle fibres in peroneus brevis (114 axons) and in peroneus tertius (82 axons) muscles. β dynamic (βD) axons are represented by filled areas, β static (βS) axons by cross-hatched areas.

Comparison of spindle β and γ supplies

During the search of β-axons, as many as possible single γ-axons were prepared and their static or dynamic action on primary ending responses to ramp-and-hold stretch was determined: sixty-two γS-axons and eleven γD-axons were identified in brevis and thirty-seven γS- and thirteen γD-axons in tertius. The number of effects they exerted exceeded their own number as most of them supplied more than one spindle.

In the thirty-five brevis spindles studied, a total of 203 effects was observed which comprised 120 γS, 31 γD, 46 βD and 6 βS effects (see Table 1 for the β effects). The proportion of βD effects (22·6 %) was of the same order as that of γD effects (15·2 %) whereas that of βS effects (2·9 %) was very small in respect to that of γS effects (59·1 %).

In the thirty tertius spindles a total of 171 effects were observed: 107 γS, 25 γD, 26 βD and 13 βS. In this muscle also the proportions of βD effects (15·2 %) and γD effects (14·6 %) were nearly the same and were comparable to those observed in the brevis. As for the βS effects, although their proportion in this muscle was greater (7·6 %) than in the brevis (2·9 %), they were also very few in comparison to the γS effects (62·5 %).

DISCUSSION

The present study supports the view that the relative importance of static and dynamic β innervation in a muscle is related to its composition in S and FR motor units: in the brevis, in which S units predominate, a large majority of dynamic β-axons was observed, whereas in the tertius, in which FR units predominate, static β-axons were as common as dynamic β-axons. A few tertius βS-axons supplied fast-fatigable units as observed by Jami *et al.* (1982).

When it was reported (Jami *et al.* 1982) that in tertius the proportion of βD-innervated spindles was lower than that observed in brevis by Emonet-Dénand & Laporte (1975), no quantitative data on the composition in motor units of peroneus brevis muscles were available and no explanation for this difference was proposed. It is now clear that it can be ascribed to the difference in motor unit composition of these muscles.

The comparison of the numbers of effects elicited by βD- and βS-axons to those elicited by γD- and γS-axons shows that the dynamic and the static β innervations do not have the same quantitative importance. The proportion of βD effects was nearly the same as that of γD effects in both muscles (around 15% of all β and γ effects), whereas γS effects were considerably more numerous than βS effects (62·5 and 59·1% as compared to 7·6 and 2·9% in the tertius and brevis respectively).

The much smaller participation of β-axons in the static control of primary ending discharges appears to be due not only to the small number of spindles (rarely more than one) supplied by individual βS-axons (see Table 1) but mostly to the intrafusal distribution of βS-axons. A βS-axon commonly supplies only one chain fibre pole, the longest, among all the several chain fibres present in a spindle, but rarely two, as shown by histophysiological studies (Harker, Jami, Laporte & Petit, 1977; Jami, Lan-Couton, Malmgren & Petit, 1978, 1979; Banks, 1991) and by tracing of the intrafusal branch of β-axons (Kucera, 1984). Thus, in a population of spindles, the total number of chain fibre poles which are supplied by γS-axons largely exceeds that of chain fibre poles supplied by βS-axons.

The situation is quite different for the βD-axons, probably also for two reasons: individual βD-axons often supply several spindles (see Table 1) and, in most spindles, there is only one fibre whose contraction increases the primary ending dynamic sensitivity, the bag_1 fibre. The same fibre is shared by β and γ dynamic axons.

This study of the β innervation in brevis and tertius peroneus muscles, in addition to showing that the difference in βS and βD innervations is likely to be related to the motor unit composition of the muscles, shows that nearly a third of motor units are β-units and that about three out of four spindles are β innervated, predominantly by βD-axons. This means that β innervation does play a significant part in the control of spindle activity and consequently in the regulation of posture and movement.

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