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Localization of the autonomic, somatic and sensory neurons innervating the cranial tibial muscle of the pig

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Summary

The location of sympathetic, somatic and sensory neurons projecting to the cranial tibial muscle of the pig hindlimb was studied with the neuronal non-transynaptic tracer Fast Blue. Additionally, the number and the size of these neurons were determinated. The Fast blue, randomly applied to the cranial tibial muscle belly of 3 pigs, labelled sympathetic neurons in the ipsilateral L5-S3 and contralateral S1 sympathetic trunk ganglia and in the prevertebral caudal mesenteric ganglia of both sides. The somatic motoneurons were identified in the ipsilateral ventral horn of the S1 segment of spinal cord, while the sensory neurons were located in the ipsilateral L7-S1 spinal ganglia. The diameter of the multipolar sympathetic neurons oscillated between 26 and 46 μm in the sympathetic trunk ganglia and between 18 and 42 μm in the caudal mesenteric ganglia. The size of the multipolar spinal motoneurons oscillated between 33 and 102 μm. The size of the pseudounipolar sensory neurons oscillated between 23 and 67 μm. In all ganglia, the labelled neurons were localized at random and did not show a somatotopic distribution. Our results document a conspicuous autonomic innervation projecting to the "classic" skeletal cranial tibial muscle. Probably this innervation is destined to the muscle vessels.

Key words

Cranial tibial muscle, pig, retrograde neuronal tracer, Fast Blue.

Key to abbreviations:

CMGs = caudal mesenteric ganglia
CTM = cranial tibial muscle
FB = Fast blue
PGs = pelvic ganglia
SC = spinal cord
SGs = spinal ganglia
STGs = sympathetic trunk ganglia

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Introduction

The skeletal muscles receive a sensory and motor innervation, that travel in the spinal nerves. In particular, studies carried out with horseradish peroxidase applied to freshly transected nerves have documented the presence of discrete numbers of sympathetic postganglionic fibres in the nerves supplying the cat (McLachlan and Jänig, 1983) and the rat (Baron et al., 1995) skeletal muscles. In spite of these observations, it is not known whether any relationship exists among the location of postganglionic neurons, the segmental spinal ganglia and the segments of spinal cord where the somatic motoneurons directly projecting to a "classic" skeletal muscle are localized. Differently our previous studies have documented the site of autonomic, sensory and somatic neurons projecting to "special" skeletal muscles associated to genital organs, such as the pig cremaster muscle (Botti et al., 2006a) and the pig bulbospongiosus muscle (Botti et al., 2009). In particular we have demonstrated a conspicuous and proportionally elevated sympathetic innervation for the aforesaid muscles.

Therefore we have undertaken a qualitative and quantitative study to identify the cell bodies of origin of the autonomic, motor somatic and sensory fibres projecting to the pig cranial tibial muscle (CTM). The neurons have been identified by retrograde labelling with Fast blue (FB), inoculated into the muscle belly.

The study was carried out in the pig, an important zootechnical animal and an interesting model in biomedical (Dodds, 1982; Swindle et al., 1992; Crissinger et al., 1994) and neuro-anatomical studies (Merighi et al., 1990; Timmermans et al., 1993; Kaleczyc et al., 1995, 1999, 2002; Kaleczyc, 1998; Majewski et al., 1999; Panu et al., 2001, 2003; Botti et al., 2006a, 2006b, 2009).

Preliminary data of this research have been performed in abstract form (Gazza et al., 2005).

Materials and methods

All procedures were approved by the local ethics committee for animal experimentation and by the Italian Ministry of Health. Precautions aimed at avoiding unnecessary suffering were taken at all stages of the experiment.

The study was carried out on the CTM of 3 intact 50 Kg b.w. pigs using the retrograde neuronal tracer method.

The animals were anaesthetized with azaperone (2 mg/Kg) and ketamine (10 mg/Kg), and their left CTM was identified by making an incision in the upper layers of the leg. In the central part of the muscle belly, 100 ml of 2% FB, a fluorescent tracer with affinity for the cytoplasm, were inoculated in five different and random sites (20 ml of 2% w/v solution per site). In all cases, the CTM fascial connective tissue represented a barrier capable of preventing the dispersion of the tracer in the surrounding tissues.

After a previously optimized 10-day survival time, the animals were again anaesthetized and intracardially perfused, first with heparinized physiological solution and afterwards with fixative solution (4% w/v of paraformaldehyde in the phosphate buffer 0.1 mol/L, pH 7.4).

The animals used in this study had 14 thoracic (Th), 7 lumbar (L), 4 sacral (S) and 20 or 22 coccygeal (Co) vertebrae.

Before collecting the samples, macroscopical and microscopical examination of the CTM and adjacent tissues revealed that the spread of FB was confined to the muscle and no evidence of the tracer in the surrounding tissues was found.

From each subject we collected, on both sides, the sympathetic trunk ganglia (STGs) from Th14 to Co1, the prevertebral caudal mesenteric ganglia (CMGs), and the pelvic plexus and its microganglia (PGs) (Panu et al., 2003), the spinal cord (SC) and the spinal ganglia (SGs) from Th14 to Co1.

The samples were preserved for 24 hours at 4 °C in the aforementioned fixative, then submitted to three washings in a phosphate buffer (0.1 mol/L, pH 7.4) and kept for 12 hours (4 °C) in the same buffer with the addition of 10% w/v sucrose. Afterwards the samples were transferred to the same buffer with the addition of 30% w/v sucrose for 72 hours (4 °C). Finally, the samples were cut into 60 μm-thick serial cryostat sections. The ganglia were cut along their longest axis and the SC was sectioned transversely. The serial sections from all samples were observed under a fluorescent microscope (Axioskop 2 plus; Zeiss, Oberkochen, Germany) equipped with epi-illumination and an appropriate UV filter set.

In order to estimate the number of retrogradely labelled neurons, all positive neurons with clearly visible nucleus were counted in each section, given that the thickness of the sections greatly exceeded the diameter of the cellular nuclei (Smolen et al., 1983). The number of the labelled cells in each site (STG, CMG, SC and SG) is presented as mean ± standard error and range.

In order to measure the neuron soma size, 10 random sections from samples containing the highest numbers of labelled cells in the different animals were projected at a magnification of 40X and photographed with a Polaroid DMC2 digital camera. The area of the perikarya was established with Simple PClp 4.0.1 image analysis software (Compix Inc., Imaging Systems, Cranberry Township, PA). No attempt to correct possible over- or under-estimation was made during the image processing, so care was taken to take measurements in identical conditions.

To better define the localization of labelled neurons in the spinal cord, the position of labelled neurons was drawn with a SPL-450 SEKONIC X-Y Plotter (Tokyo, Japan).

Results

Distribution and frequency of labelled cells

The use of the retrograde neuronal tracer allowed us to locate CTM-projecting neurons in STGs, CMGs, SC and SGs (Fig. 1).

The labelled cells of the sympathetic trunk were constantly found in the ipsilateral L5-S3 and contralateral S1 ganglia. The number of STGs perykaria was 1593.67±60.6 (range 1521-1714), the vast majority of them (1527.67±65.07, range 1430-1651) was located in the ipsilateral ganglia.

Positive neurons were also found in CMGs (132.67±12.91, range 107-148) bilaterally. The vast majority of these cells (109.33±10.04, range 96-129) was located in the ipsilateral ganglion.

Labelled perykaria were found in the SC, where they found in the ipsilateral ventral horn of S1 segment. The labelled motoneurons were 88.67±3.38, range 82-93.

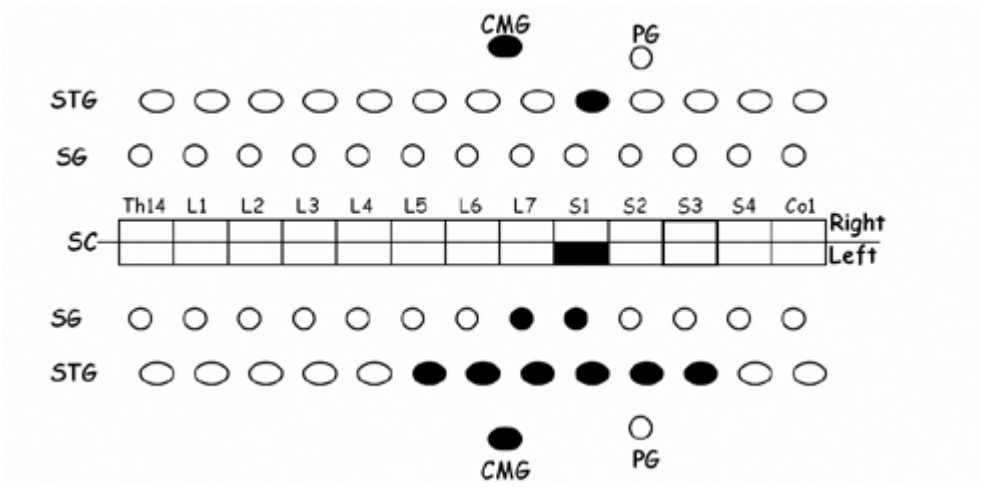

Figure 1 – Schema of the distribution of labelled neurons in the (STG), caudal mesenteric ganglia (CMG), spinal ganglia (SG) and spinal cord (SC) after injection of Fast blue in the left cranial tibial muscle.

Retrograde labelled sensory neurons were found in the ipsilateral L7-S1 SGs. The number of sensory cells was 46.67±3.18, range 41-52.

In all autonomic and sensory ganglia, the labelled neurons did not show any preferential localization which could suggest a somatotopic distribution.

Morphometric characteristics and diameters of autonomic, somatic and sensory neurons

The STGs neurons were multipolar and their diameter oscillated between 26 and 46 μm (Fig. 2).

Also the CMGs neurons were multipolar with a diameter oscillating between 18 and 42 μm (Fig. 3).

The positive neurons of SC were multipolar with a diameter oscillating between 33 and 102 μm (Fig. 4).

The sensory CTM-projecting neurons were roundish and devoid of dendrites, with a diameter varying between 23 and 67 μm (Fig. 5).

Discussion

The present study, for the first time in a breeding species, yields information on localization, number and size of the postganglionic, somatic and sensory neurons projecting to the pig CTM, a "classic" hindlimb skeletal muscle. After application of FB to the muscle belly, labelled cell bodies were identified bilaterally in STGs and in CMGs, and only ipsilaterally in SC and SG.

The vast majority of CTM-projecting neurons were autonomic. They were concentrated in the sympathetic chain ganglia, with a broad cranio-caudal extension, and in

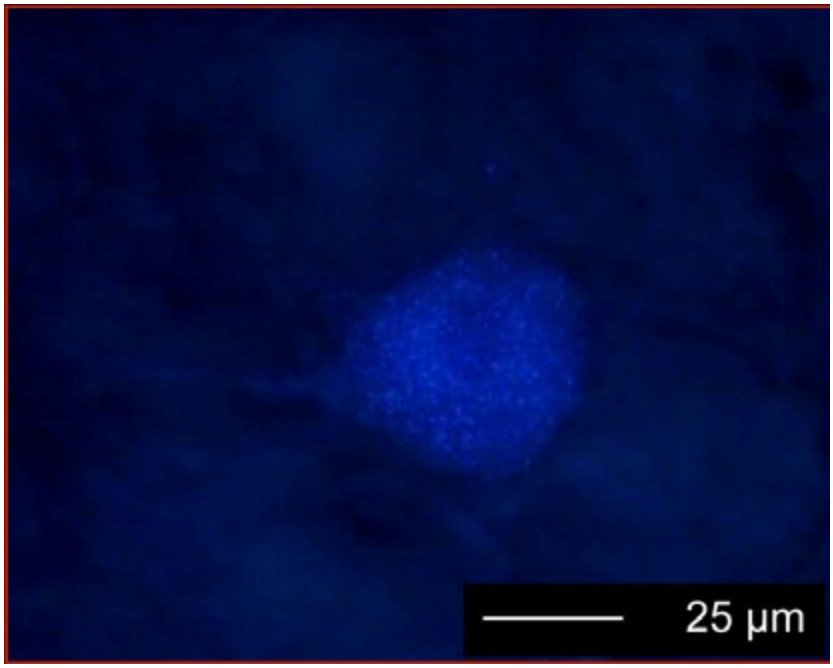

Figure 2 – Micrograph showing a multipolar labelled neuron of the left, ipsilateral, L7 sympathetic trunk ganglion. Bar = 25 μm.

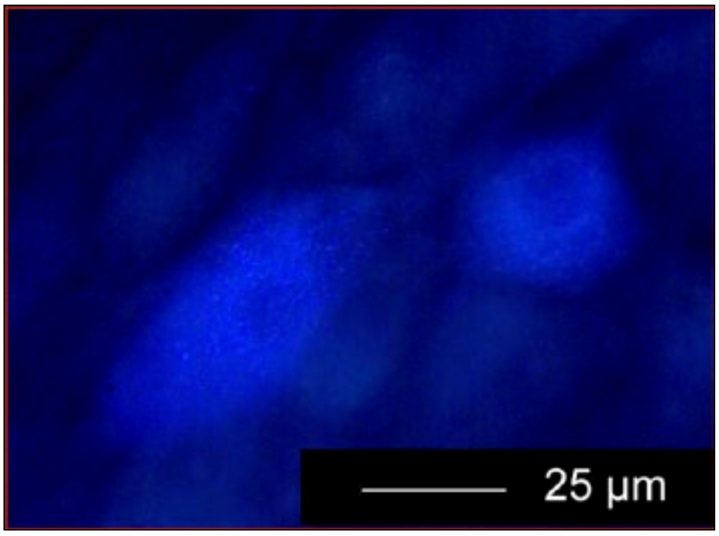

Figure 3 – Micrograph showing two multipolar labelled neurons of the left, ipsilateral, caudal mesenteric ganglion. Bar = 25 μm.

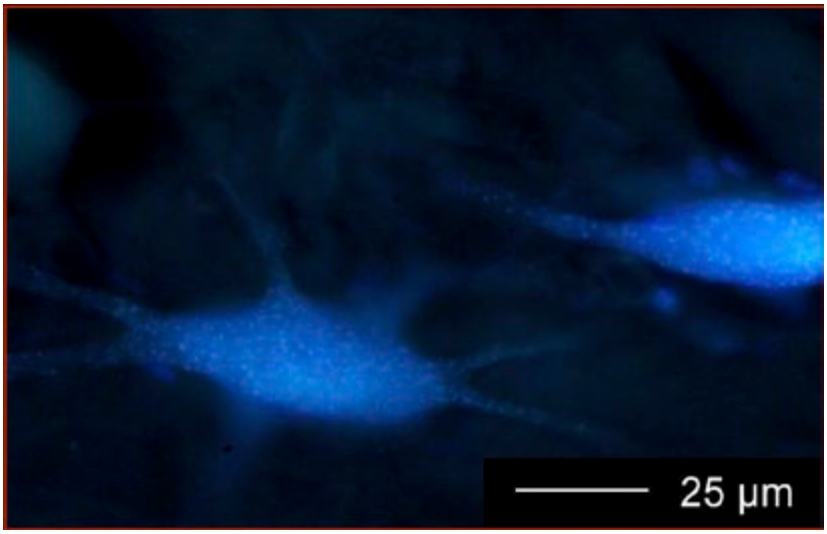

Figure 4 – Micrograph showing two multipolar somatic labelled neurons of the S1 segmant of the spinal cord. Bar = 25 μm.

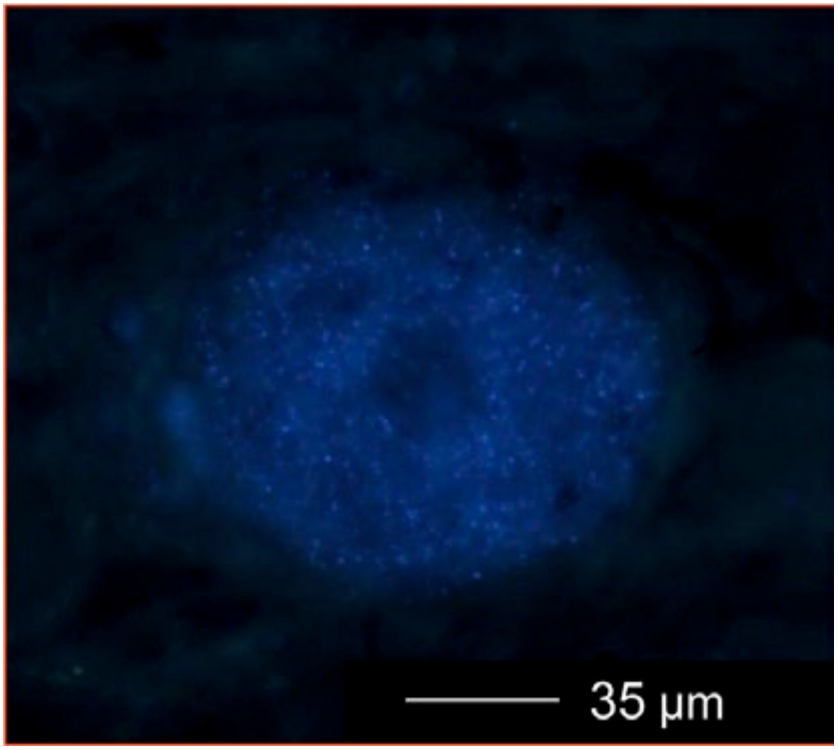

Figure 5 – Micrograph showing a labelled pseudounipolar neuron of the left, ipsilateral, S1 spinal ganglion, projecting to the cranial tibial muscle. Bar = 35 μm.

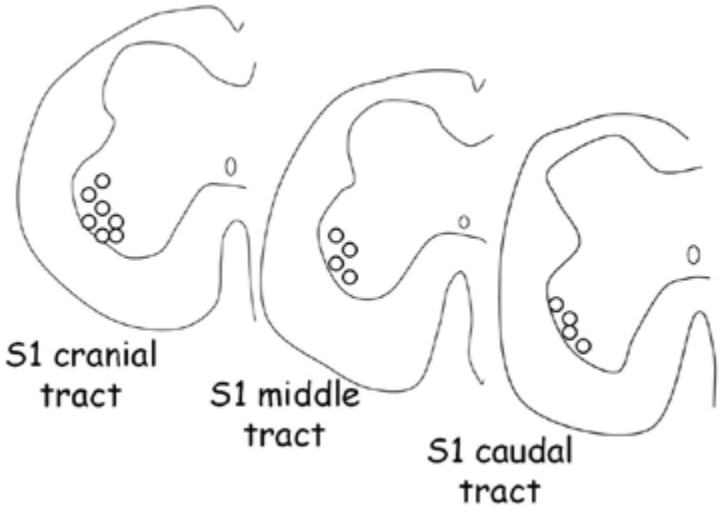

Figure 6 – The localization of labelled spinal motoneurons following Fast blue injections into the cranial tibial muscle is shown in tranverse sections of 3 different tracts of the S1 segment of the spinal cord.

the CMGs. Therefore, we retain that the autonomic source of porcine CTM innervation is sympathetic in nature.

We consider that the remarkable autonomic innervation of the pig CTM could be attributed, primarily, to the vascular contingent of the muscle, as it has already been demonstrated for the vessels of the guinea-pig limb muscles (Grasby et al., 1997) and human skeletal muscle (Saito et al., 1997). Autonomic control of vasomotor functions regulates muscle metabolism (Grant, 1966; Baez, 1973; Fleming et al., 1987, 1989; Franken et al., 1996; Berg et al., 1997; Kurjiaka, 2004). In fact, during skeletal muscle contraction it is possible to observe a functional vasodilation, rapidly followed by a sympathetic mediated vasoconstriction (Thomas and Segal, 2004). Initially the rhythmic skeletal muscle contractions augments capillary perfusion (Sweeney and Sarelius, 1989) and promotes the extraction of oxygen and nutrients from the blood (functional vasodilation) (Gorczynski et al., 1978; Marshal and Tandon, 1984). Subsequently the blood flow is restricted by the autonomic nervous system to increase and preserve the local pressure (Van Teeffelen and Segal, 2003).

Furthermore, an autonomic innervation could facilitate the neuromuscular transmission produced by an increased release of acetylcholine (Kuba and Tomita, 1971), and either prolong or curtail the active state of motor units (Barker and Saito, 1981).

Our research allowed to localize the pig CTM motor somatic neurons. These cells were located ipsilaterally in the ventral horn of S1 segment of SC. The motoneurons are distributed in all the lateral part of the ventral horn, even if the vast majority of labelled somata were topographically located in the lateral part of the apex of ventral horn (Fig. 6).

The primary sensory neurons of the CTM were located in the ipsilateral L7-S1 SGs. The lumbo-sacral extension of marked SGs found in this study confirms that

also in the pig the afferent fibres from CTM travel along the deep peroneal nerve, that is a branch of the common peroneal nerve and this is a branch of the ischiatic nerve. In the pig the ischiatic nerve originates from L6 and S2 nerves (Getty, 1982; Nickel et al., 1988). The afferent projections from the CTM may transmit different somatic and visceral inputs to the spinal cord. This information, which originates from the striated fibres and smooth vascular musculature of the CTM, could influence muscle contraction and metabolism.

Our study, carried out by the retrograde neuronal tracer technique, has localized the autonomic, somatic and sensory neurons innervating a pig hindlimb muscle, the CTM, and has documented that the vast majority of the neurons projecting to the muscle and its blood vessels are autonomic.

Further research needs to be carried out in order to define the neurochemical content of the neurons projecting to the CTM.

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