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Distribution and morphology of the NOS-immunoreactive neurons in the thoracolumbar and sacral spinal cord of the pig

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Summary

The study investigated the distribution and morphology of nitric oxide synthesis-immunoreactive neuronal cell bodies and processes in the thoracolumbar and sacral spinal cord of sexually immature gilts. Investigations revealed the following: NOS-immunoreactive fibers and singular cell bodies in regions of the dorsal horn including superficial laminae I and II and deeper laminae III and IV; prominent NOS-immunolabeled perikarya in intermediolateral nucleus of the thoracolumbar spinal segments; NOS-positive perikarya in lamina X along the thoracic, lumbar and sacral divisions of the cord; NOS-positive perikarya and fibers in the area near to the location of the intermediomedial and intermediolateral nuclei in the sacral segments of the cord. The obtained morphological results indicate that the general distribution pattern of NOS-positive neurons in the spinal cord of pigs resembles that of other species. The concentration of NO-ergic neurons in the autonomic nuclei, dorsal horn laminae I, II, III, IV and lamina X suggests a prominent role of NO-ergic neurons in visceral and sensory functions.

Keywords: NOS-immunoreactivity, spinal cord, pig

Nitric oxide (NO), an active radical acts as a messenger of interneuronal information (2, 4). Following synthesis the NO diffuses to adjacent neuropil affecting vicinal neurons (9). In the nerve cells three isoforms of the NOS enzyme have been identified (3). All of them, neuronal NOS (nNOS), endothelial (eNOS) and inducible (iNOS) express enzymatic activity of NADPH-diaphorase which has been commonly used as a histochemical marker for NOS (17).

Previous histochemical studies have identified the NADPH-d activity among others in spinal cords of the rat (18, 1), rabbit (13), cat (19), dog (20) and human (8). Consequent application of anti-NOS antibodies for visualization of nitric oxide synthase enabled immunocytochemical detection of the NOS-positive neurons in the spinal cord of the rat (6, 7), mouse (6), guinea pig (5), cat (6, 19), dog (15) and squirrel monkey (6). Although available reports disclosed structure of the NO-ergic system in the spinal cord of mostly laboratory and companion animals the distribution and morphological details of NO-ergic system in large husbandry animals still have not been studied. In fact, pig due to its embryological, anatomical and physiological similarities to human constitutes especially valuable species for bio-medical research (16). Currantly, however, the precise anatomical characteristic of NO--ergic neurons in the porcine spinal cord remains still unknown.

The present study have been undertaken to investigate morphology and distribution of the NO-ergic neurons in the thoracolumbar and sacral spinal cord of the pig with application of the specific anti-nNOS antibody.

Material and methods

All experimental procedures were in agreement with the Polish principles of laboratory animal care (NIH publication No. 86-23, rev. 1985) and the specific national laws on experimental animal handling.

Three sexually immature gilts of the Large White Polish breed (body weight ca 20 kg) obtained from the commercial fattening farm were used for the study. All the animals were deeply anaesthetized with pentobarbital (Vetbutal, Biowet, Poland, 30 mg/kg of body weight) and perfused transcardially with 4% solution of paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). After perfusion spinal cords were collected from all the animals studied, postfixed in the same fixative as used for perfusion (2 hours),

rinsed in PB overnight and finally transferred to and stored in 18% buffered (pH 7.4) sucrose solution until further processing. Then transverse frozen sections of selected spinal segments (Th1, Th7, Th13, L2, L5, S1, S2) were cut with cryostat at the thickness of 20 μm. Serial sections were mounted on chrome alum-gelatine-coated slides and air-dried.

Sections were subjected to single immunostaining for nNOS as described earlier (12). The slides were then analyzed under a fluorescent microscope (Axiophot, Zeiss, Germany) and photographed with the confocal microscope (BIO-RAD). Omission of the primary antisera as well as their replacement with normal mouse serum proved the specificity of the immunoreaction.

Results and discussion

Neuronal perikarya and processes expressing NOS immunoreactivity were found at all segmental levels throughout studied regions of the porcine spinal cord (fig. 1). The stained elements were encountered in the gray matter. However, the white matter was devoid of stained cells, bundles of the NOS-positive fibers penetrating the border zone of the dorsal horn were observed (fig. 2a). The NOS-immunoreactive fibers and singular cell bodies were observed in regions of the dorsal horn including superficial laminae I and II and deeper laminae III and IV. Prominent perikarya were disclosed in intermediolateral nucleus in the thoracolumbar spinal segments. They were found also in laminae X along the thoracal, lumbar and sacral divisions of the cord. Additionally, a concentration of the NOS-positive perikarya and fibers constituting intermediomedial nucleus was observed in the sacral segments of the cord.

Depending on the cross-section level the dorsal horn exhibited differential concentration and location of the NOS-immunopositive fibers. At the level of Th1 and Th7 the dense plexus of tiny varicose fibers was observed in the laminae I and II (fig. 2b). At Th13 level the stained fibers appeared additionally at lamina III of the horn, while caudally at level L2 the zone of the stained fibers extended to lamina IV. Beginning from L5 cross-section the area of appearance of the NOS--stained fibers reduced backward reaching the extent of laminae I, II and III at S1 level. Light microscopic examination revealed NOS-immunoreactive cell bodies at different locations of the stained laminae (fig. 2c). Thoracic segments Th1-Th13 disclosed incidentally distributed, single labeled perikarya interposed between the stained processes. In dorsal horn of the lumbar segments L2 and L5 a higher number, up till 5 per stained area, of the NOS-positive cell bodies was observed. Caudally, at S1 plane section a number of stained perikarya comparable to lumbar division was located between labeled fibers. Neurons of the dorsal horn (fig. 2d) possessed round or spindle shaped perikarya filled with intensely, moderately or weakly stained cytoplasm. Ring of the stained cytoplasm surrounded oval, centrally located, unstained nucleus. The diameter of perikarya varied since 10 to 15 μm. Occasionally, fragments of thicker processes running between tiny fibers were observed (fig. 2e).

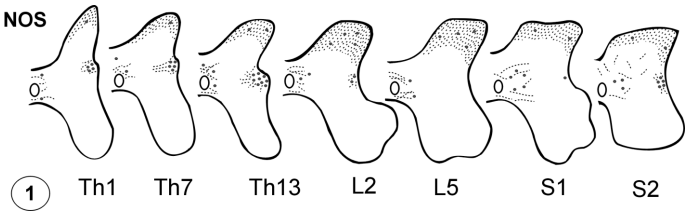
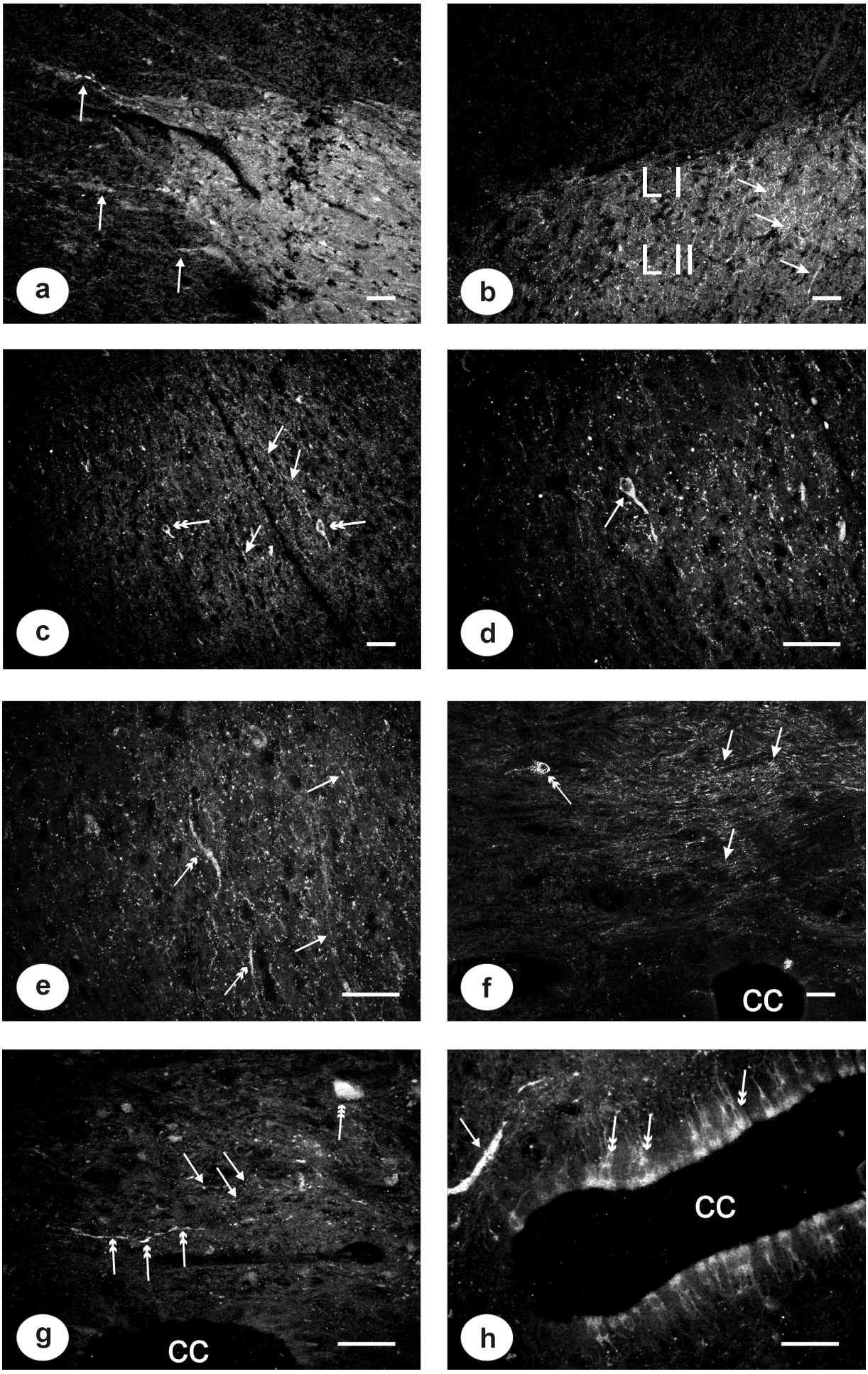


Fig. 1. Distribution of positively labeled nerve cells (bold dots) and fibers identified by NOS-immunocytochemistry in selected spinal segments (Th1, Th7, Th13, L2, L5, S1, S2) of the thoracolumbar and sacral spinal cord of the pig. The drawings were prepared by averaging the counts from three pigs

Through the length of all studied segments (Th1-S2) individual stained cells were found in the area around the central canal (fig. 2f). The moderately and intensely stained neurons exhibiting oval, triangular and fusiform perikarya measured 15 till 25 μm in diameter. The space between loosely arranged perikarya was occupied by two types of neuronal processes penetrating the matrix in majority of cases horizontally (fig. 2g). The tiny varicose fibers were intermingled with thick non varicose processes resembling fragments of separated proximal neuronal protrusions. Individual stained cell bodies dispersed in the gray matter towards the intermediolateral horn were also occasional encountered. The specific NOS immunofluorescence appeared in ependymal lining filling cytoplasm of tanycytes surrounding the central canal (fig. 2h).

The intermediolateral nucleus contained concentration of the NOS-immunopositive cell bodies and fibers through Th1 till L2 spinal segments (fig. 3a). The neurons were scattered throughout the nucleus reaching maximal number of about 10 perikarya per nuclear region at the Th13 level of the cord. The perikarya of oval, triangular and spindle morphology measured about 30-35 μm in diameter. The cells displayed oval nuclei surrounded by a band of well stained cytoplasm. The neural somata send pronounced horizontal projections oriented laterally towards spinal white matter and medially towards gray matter of laminae X. However, the medial fibers terminated in half way between intermediolateral horn and central canal of the cord (fig. 3b). The projections resembled portions of the peripheral fragments of the neuronal somata filled with immunoreactive cytoplasm.

In the sacral segment S1 at the region of intermediomedial nucleus, a group of scattered NOS-immunoreactive perikarya was found (fig. 3c). The stained perikarya were distributed in a ring like structure at the border zone of the nucleus. Although morphology of the neurons resembled those of the intermediolateral nucleus of the thoracic division, they were 25-30 μm in diameter and number of the cells was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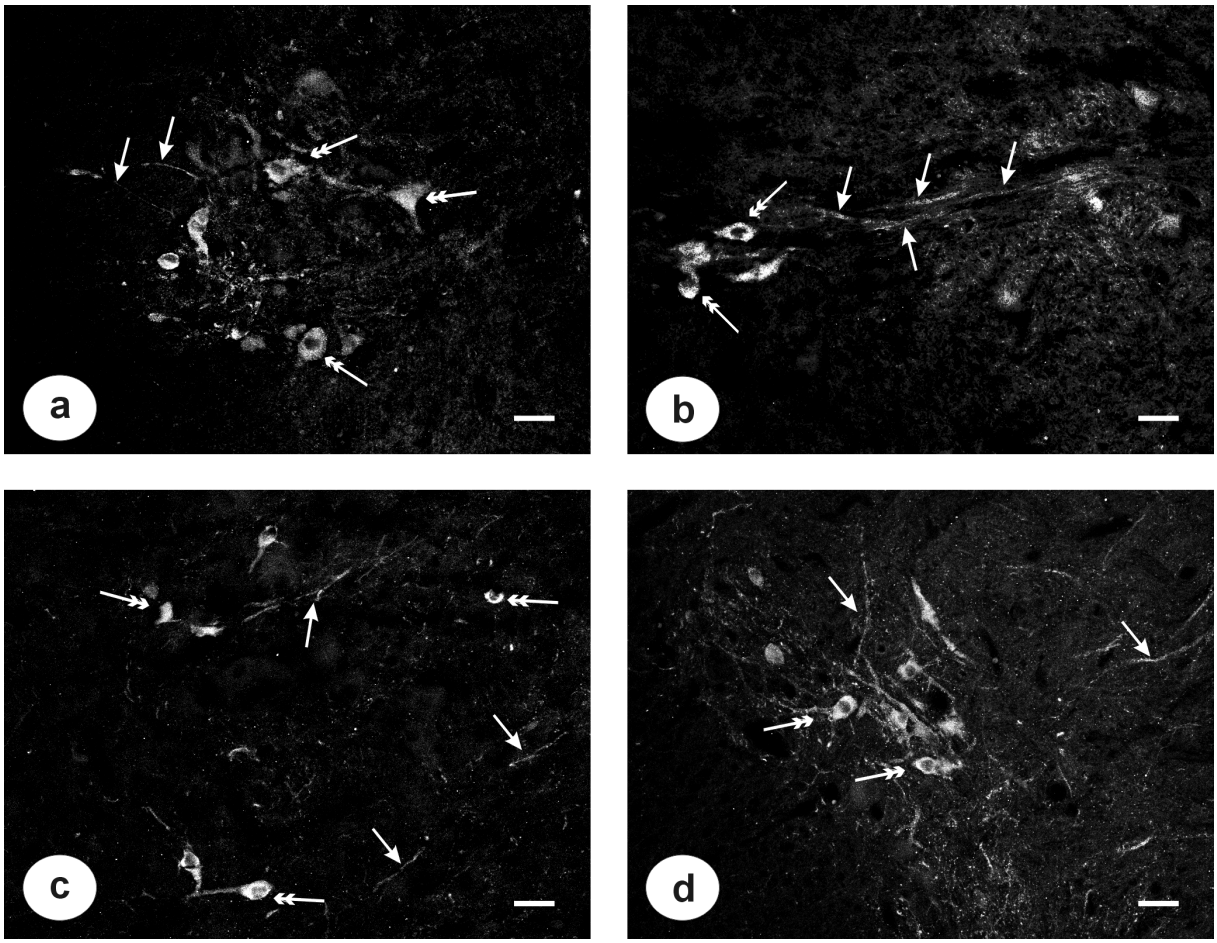

Fig. 3. (a) NOS-immunopositive cell bodies (double arrows) and fibers (single arrows) in the intermediolateral column of the gray matter at the Th13 level of the cord. (b) Horizontal neuronal projections (single arrows) oriented medially in the gray matter sent by neuronal somata (double arrows) of the intermediolateral nucleus at the level Th7 of the spinal cord. (c) A group of scattered NOS-immunoreactive perikarya (double arrows) and fibers (single arrows) located in the outer zone of the intermediomedial nucleus in the S1 sacral segment. (d) A group of NOS-positive neurons (double arrows) and fibers (single arrows) in intermediolateral nucleus at S2 level of the spinal cord. Bar = 40 µm

smaller (about 4-6 per nucleus). Between the stained cell bodies moderate number of thick non varicose processes was observed. At this segmental level only single cell bodies in intermediolateral nucleus were found.

Backward, at S2 level the intermediomedial nucleus contained only 2-3 NOS-positive perikarya, while at intermediolateral nucleus number of the stained cells increased up to 6 gathered together perikarya per nuclear region (fig. 3d). Between the cell bodies the NOS--immunoreactive processes were observed.

The present study for the first time has identified distribution and morphology of the NOS-immunoreactive cell bodies and fibers in the thoracolumbar and sacral spinal cord of the pig. In our study, a numerous population of dorsal horn fibers occupying laminae I and II in all studied segments and additional laminae III in Th13 and IV in L2 and L5 segments was found. The stained laminae contained NOS-immunoreactive neuronal cell bodies occasionally interposed between the fibers. However, the NOS staining pattern of the porcine spinal cord was consistent with previously stu-

Fig. 2. (a) NOS-immunoreactive fibers (arrows) penetrating the border zone of the dorsal horn at L5 segment of the porcine spinal cord. (b) The dense plexus of tiny varicose fibers (arrows) in the laminae I (LI) and II (LII) in Th7 segment of the cord. (c) NOS-immunoreactive cell bodies (double arrows) intermingled with NOS-positive fibers (single arrows) at different locations of stained laminae of the L2 level of the dorsal horn. (d) Spindel shaped cell (arrow) of the dorsal horn filled with intensely stained cytoplasm. (e) Thick NOS-positive processes (double arrows) running between tiny NOS-immunoreactive fibers (single arrows) in the dorsal horn at the L5 segment. (f) Individual stained cell (double arrow) and the plexus of the tiny varicose fibers (single arrows) in the area around the central canal (cc) at the L5 level. (g) Thick non varicose process (double arrows) penetrating the matrix of the lamina X horizontally, intermingled with tiny varicose fibers (single arrows), and single stained cells (triple arrow). (h) Specific NOS immunofluorescence in cytoplasm of tanacytes (double arrows) surrounding the central canal (cc) and process of the nerve cell (single arrow) resembling fragment of separated proximal protrusion in the lamina X at the S2 level of the porcine spinal cord. Bar = 40 µm

died in the guinea pig (5), cat (19) and rat, cat, mouse and squirrel monkey (6), the number of NOS-positive cells in the monkey superficial layers was conspicuously less than in the same areas of rodent and feline dorsal horn. The observed discrepancy may reflect possible interspecies differences. Overall, the distribution of NOS-immunoreactivity in the pig dorsal horn laminae suggests that NO may be involved as a modulator or neurotransmitter in afferent pathways (14).

The present investigation revealed prominent NOS--immunolabeled perikarya in intermediolateral nucleus of the pig. This localization of NO-ergic neurons is congruent with earlier reports in rat (7), guinea pig (5), cat and squirrel monkey (6). In the rat (10) and cat (11) some NO-ergic neurons were also found in dorsal commissural nucleus, which was shown to contain a population of sympathetic preganglionic neurons. Our results show that sympathetic preganglionic neurons in the pig are likely to synthesize and release NO, however, deficiency of NO-ergic perikarya in the porcine dorsal commissural nucleus indicate possible interspecies differences in spinal distribution of the sympathetic neurons.

In lamina X of the porcine spinal cord, throughout its thoracic, lumbar and sacral divisions, singular NOS--immunoreactive neuronal cell bodies were identified. Although topography as well as morphology of NOS--positive neurons of this region is generally consistent with other studied species a visible quantitative differences can be noticed. In the pig, like in the cat and squirrel monkey (6, 19) the number of NOS-immunoreactive cells in lamina X appeared to be smaller as compared to that in the rat and mouse spinal cord (6). Moreover, in the rat and guinea pig (5) the NO-ergic perikarya of laminae X were found to be concentrated in dorsal commissural nucleus, while in the pig this nucleus contained single NOS-positive cells or even was devoid of NO-ergic neurons. In the rat cells of lamina X are likely to be central sympathetic preganglionic neurons (10), although in the pig, both, projections as well as target sites of NOS positive cells in lamina X remain unknown.

In the porcine sacral spinal cord of S1 level a few NOS-positive neurons were found in the intermediomedial nucleus. Earlier studies on guinea pig (5) and cat (19) revealed only singular NO-ergic neurons in the lateral horn while region of the intermediomedial nucleus was devoid of NO synthesizing cells. However, backward at S2 porcine segmental level the NO--ergic parasympathetic cell bodies were encountered simultaneously at the intermediomedial and intermediolateral nuclei. Thus our observations indicate interspecies differences in distribution of sacral parasympathetic neurons, defining in the pig their two intermediomedial and intermediolateral locations, while in guinea pig (5) and cat (19) the neurons were found exclusively in intermediolateral sacral horn. Moreover, expression of nitric oxide synthase in the porcine parasympathetic neurons provides an indication that NO may play a key role in porcine sacral parasympathetic neurons.

Thus, our morphological results concerning distributional structure of NOS-positive neurons in the thoracolumbar and sacral spinal cord indicate that in spite of interspecies differences, the general pattern of distribution of NOS-positive neurons in the spinal cord of the pig resembles that of other species. The concentration of NO-ergic neurons in the autonomic nuclei, dorsal horn laminae I, II, III, IV and lamina X suggests a prominent role of NO-ergic neurons in visceral and sensory functions.

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