Difference of Pericentral NADPH-d Positive Neurons in the Rabbit Spinal Cord Segments

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Abstract. The purpose of the present investigation was to characterize and determine the number of NADPH-diaphorase positive neurons around the central canal in the rabbit spinal cord These neurons are known to function as interneurons and are present in all spinal cord segments They differ in shape of their bodies and in length and branching of their processes The main differentiation was observed in their number, depending on the place of their localization The highest number of these NADPH-diaphorase positive neurons was in sacral part (6 in average), the lowest one was noticable in thoracic spinal cord (1–2 in average) It can be concluded that pericentral neurons of the rabbit spinal cord are capable of synthesizing nitric oxide and that they differ in number, depending on the place of their localization in each spinal cord segment

Key words: NADPH-diaphorase — Pericentral — Spinal cord

The histochemical NADPH-diaphorase (NADPH-d) reaction has identified distinct neuronal populations in the nervous system of several species (Bredt *et al* 1991) Considerable evidence suggests that NADPH-d is a neuronal nitric oxide synthase (NOS), which indicates the presence of nitric oxide (Hope *et al* 1991) NADPH-d positive neurons have been shown to exist in the spinal cord, the staining was present in the superficial dorsal horn and in neurons around the central canal at all spinal levels (Valtschanoff *et al* 1992a) In addition, major cell groups were identified in the intermediolateral (IML) cell column of the thoracic and sacral levels (Vizzard *et al* 1997) NADPH-d neuronal populations of dorsal horn and IML have generated considerable interest regarding their physiological functions (Tang *et al* 1995, Valtschanoff *et al* 1992b) The present study was undertaken to ascertain the involvement of nitric oxide in neurons of pericentral region with the special interest regarding their difference in all of segments throughout the spinal cord

Adult rabbits of both sexes (250-350 g) were anesthesized with pentobarbital (30 mg/kg, i v) and perfused transcardially with saline followed by freshly prepared 4% paraformaldehyde + 0 1% glutaraldehyde buffered with 1mol/l sodium phosphate, pH 7 4 Fixation procedure, sectioning and storage of sections as well as NADPH-d histochemical detection were performed as it is reported in our previous studies (Maršala *et al* 1997, Kluchová and Dorko 1997) NADPH-d staining in cells and fibers could be identified

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around the central canal (lamina X) throughout the rostrocaudal direction of the spinal cord But, there could be seen some differences in morfology and mainly in number in some spinal cord segments The cervical spinal cord revealed higher number of NADPH-d positive pericentral neurons in rostral part There could be seen about 7 neurons in average in transverse sections In caudal direction this number decreased to 2 cells in one section (Tab 1) They were triangular in shape, with the processes directed to ventral, dorsal and lateral side of gray matter High density of body staining could be seen in all cervical segments

Table 1. The average number of NADPH-d positive neurons of lamina X in cervical, thoracic, lumbar, sacral and coccygeal spinal cord segments

Segments	1	2	3	4	5	6	7	8	9	10	11	12
Cervical	6 75	5 82	5 55	4 62	3 92	3 08	3 00	2 13				
Thoracic	2 34	1 56	1 42	2 00	2 33	2 00	1 80	1 67	2 00	1 87	2 14	1 69
Lumbal	2 00	2 34	3 03	3 84	4 16	3 80	3 80					
Sacral	4 94	5 89	4 59	3 77								
Coccygeal	2 50											

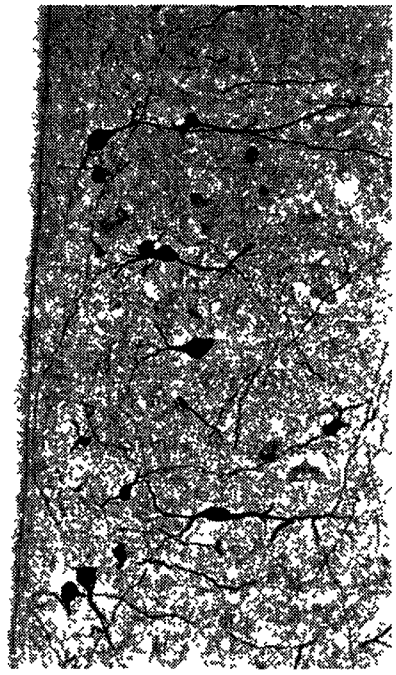

Figure 1. Morphological features of NADPH-d positive neurons in lamina X of the sacral spinal cord in longitudinal section

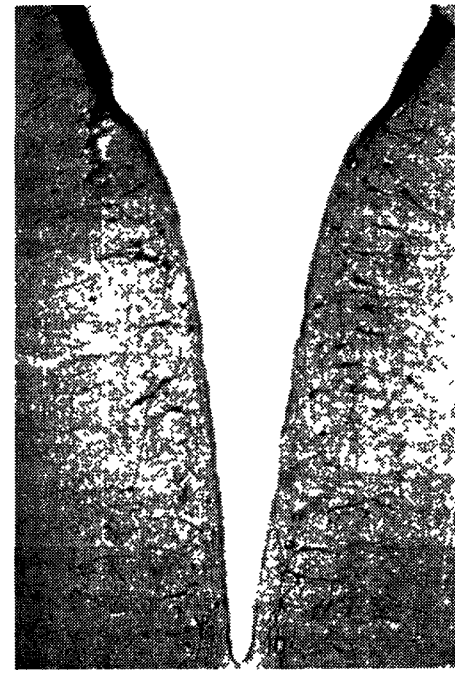

Figure 2. Longitudinal section through the pericentral zone Note the number of NADPH-d positive cells in sacral (above) and coccygeal (down) spinal cord

Number of pericentral NADPH-d positive neurons rapidly decreased to 1 5–2 cells in the average in all thoracic segments These neurons were located more away from the ependymal layer of the central canal The direction of their processes was mainly lateral

In lumbar spinal cord NADPH-d positive neurons were irregular in shape with the rich process arborization, especially seen in the lumbar intumescence Processes were oriented less to the lateral side, they run deeper to the ventral horns Number of NADPH-d positive cells in lamina X was increasing in rostrocaudal direction (Tab 1)

Positively stained pericentral neurons in sacral and coccygeal segments differed in shape in comparison with other parts of spinal cord Intensively stained round or oval cells were markedly seen (Fig 1) They had long processes almost without arborization, extending throughout most of the spinal gray matter In S2 segment, in average about 6 NADPH-d positive pericentral neurons were seen They decreased in number in caudal direction (Fig 2) Their distance from ependymal layer was evident In caudal sacral and in coccygeal segments these neurons not only decreased in number but reduced intensity of staining could be also seen

In this study we have demonstrated the difference in the number of pericentral NADPH-d positive neurons which can be dependent on the amount of the gray matter in the corresponding spinal cord segment The presence of NADPH-d staining suggests that these neurons are involved in the utilization of nitric oxide

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