

Selective Sparing of NADPH-d Positive Spinal Cord Neurons Affected by Ischemia

D KLUCHOVÁ AND J MARŠALA

Department of Anatomy, Medical Faculty of Šafárik University, Košice, Slovakia

Abstract. Histochemical characterization of NADPH diaphorase positive neuronal pools in the rabbit lumbosacral segments was performed during and after transient spinal cord ischemia. Strongly enhanced staining of NADPH diaphorase positive structures appeared in the superficial dorsal horn, the pericentral region and in the neurons of the sacral parasympathetic nucleus at the end of 40 min of abdominal aorta ligation or after 1 day reperfusion. Four days after ischemia, NADPH-d positive neurons and vessels were detected in the central gray matter despite well developed necrosis in this location. Regional nitric oxide synthesis and its vasodilatory effect during the period of aortic occlusion may account for the observed selective resistance of these spinal cord neurons to transient ischemia.

Key words: Ischemia — NADPH-diaphorase — Spinal cord

The role of NO (nitric oxide) was in recent years studied by many authors during various pathological conditions. The aim of this work was to study the influence of ischemia on NO-containing neurons in the spinal cord. Spinal cord ischemia caused by aortic occlusion may evoke neuronal degeneration of spinal cord neurons. Previous studies have demonstrated models in which aorta was occluded, causing a significant reduction in spinal cord blood flow and corresponding spinal neuronal degeneration during reperfusion (Chavko *et al* 1991, Maršala *et al* 1994). Histopathological changes in such paraplegic animals show typical affection of neuronal pools localized between laminae IV and VII (Zivin *et al* 1982). Our interest was focused on the presence of NADPH diaphorase (NADPH-d) in the spinal cord affected by ischemia, which can reflect nitric oxide synthesis (Vincent and Kimura 1992). The localization of NADPH-d positive neurons in the spinal cord appears to correlate with the areas typically found to be resistant to spinal cord ischemia (Anderson 1992, Valtschanoff *et al* 1992).

Adult rabbits of both sexes (2.5–3.0 kg) were anesthetized with pentobarbital (30 mg/kg, i.v.). A small subcostal incision was performed, a snare ligature with a long file was then placed around the aorta and tightened for 40 min. Before induction of the ischemia the animals were divided into four groups: 1) 40 min abdominal aorta ligation (no perfusion) followed by perfusion fixation, 2) 40 min abdominal aorta ligation followed by 1 day of reperfusion, 3) 40 min abdominal aorta ligation followed by 4 days of reperfusion, 4) sham-operated nonligated control. At the end of protocols perfusion fixation was

Correspondence address: D Kluchová, Department of Anatomy, Medical Faculty of Šafárik University, Šrobárova 2, 041 80 Košice, Slovakia. E-mail: kluch@dodo.sk

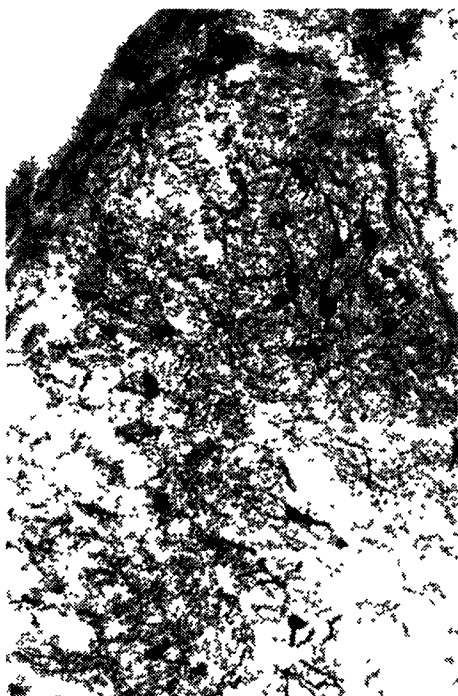


Figure 1. Strongly enhanced staining of small NADPH-d positive neurons with the punctate NADPH-d positive staining in the superficial dorsal horn (laminae I-III) 1 day after 40-min ischemia

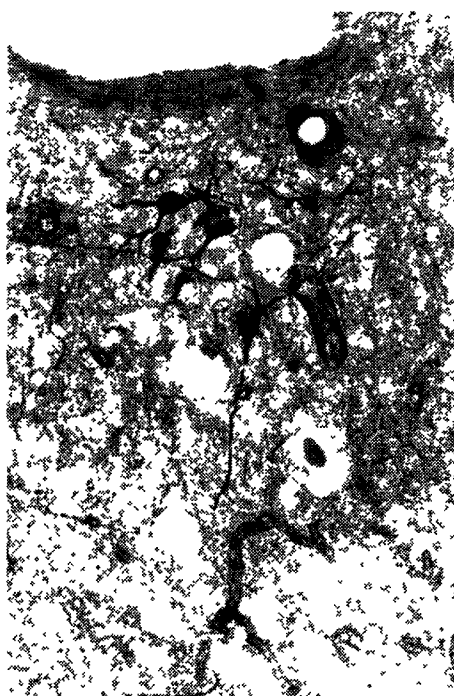


Figure 2. NADPH-d positive neurons of the pericentral zone with their processes extending laterally to the necrotic field on the 4th day of postischemic period

performed Fixation procedure, sectioning and storage of sections, as well as NADPH-d histochemical detection were described in our previous studies (Maršala *et al* 1997, Kluchová and Dorko 1997)

The topographical distribution of NADPH-d positive neurons in lumbar and sacral segments of sham - operated animals showed that the majority of NADPH-d neurons were located in the superficial dorsal horn (laminae I-III), in the pericentral area (lamina X) and in the intermediolateral cell column. A 40-min ischemia and immediate perfusion fixation did not influence the intensity of NADPH-d staining of any neuronal population in lower lumbar segments. Occasionally, in S2-S3 segments some elongated NADPH-d positive neurons of SPN were found more ventrally, forming a narrow cellular band along the posterolateral periphery of the anterior horn. As a consequence of 40-min abdominal aorta cross-clamping and 1 day survival, highly expressed staining of all components of NADPH-d positive populations occurred. The most prominent NADPH-d staining was detected in the superficial dorsal horn layers (Fig 1). Fully developed neuropathological damage of lower lumbar and all sacral segments occurred after 4 days reperfusion following 40-min ischemia that caused the formation of large necrotic foci encompassing the central



Figure 3. View of the necrotic field 4 days after ischemic insult. Positively stained cell bodies, their processes and vessels can be seen

spinal cord gray matter (lamina VII) In spite of the large extent of necrosis, the integrity of the pericentral gray matter (lamina X) and superficial dorsal horn layers (laminae I-III) remained intact (Fig 2) Large multipolar neurons in deeper layers of dorsal horn appeared unchanged Their long branches often extended deeply into the necrotic field, where a large number of strongly enhanced staining of NADPH-d positive vessels could be seen (Fig 3) An apparently decreased NADPH-d staining was noted at the S2 segment level in the region of sacral parasympathetic nucleus (SPN)

In the present study we have demonstrated that an injurious interval of spinal ischemia (40 min) after 4 days reperfusion evokes the development of gray matter necrosis in LS spinal cord segments seen mostly in lamina VII However, almost complete resistance of neuronal pools in the superficial dorsal horn (laminae I-III) and in lamina X was detected It can be concluded that local NO synthesis and its direct vasodilatory effect during the period of spinal cord ischemia may account for the selective resistance of these neurons to the transient aortic occlusion

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