Coexistence of Nitrergic and Acetylcholinesterase (Ache)-Positive Nerve Structures of the Phaesant Spleen

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Abstract. The coexistence of neuronal NADPH-diaphorase and ACHE activities were investigated in the phaesant spleen by succesive double histochemical staining of the same sections Two types of nerve structures were found in pheasant the spleen nerve cells and nerve fibres NADPH-d and ACHE-positive nerve fibres in colocalization enter the spleen in its hilum in the vicinity of splenic artery branches and are gradually distributed in periarterial topography in the white pulp Only NADPH-d positive nerve cells were seen around the splenic vessels In the red pulp and splenic capsule, only ACHE-positive nerve fibres were present

Key words: Spleen — NADPH-diaphorase — ACHE — Nerve structures

Spleen, a secondary lymphopoetic organ playing an important part in immunological reactions, is under the influence of a neuronal regulation Distribution of intrasplenic nerves and their eventual role in the spleen function has been studied by several authors Several suggestions indicate that innervation of primary and secondary lymphatic organs provides the anatomical, physiological and neurochemical basis for interactions between the nervous and immune systems (Bulloch 1985) While the species-specific differences of innervation in the mammalian spleen are well documented, a little attention has been paid to the study of innervation in lower animals spleens (Schmidtová *et al* 1995, 1998) The immune functions are also regulated by other peptidergic and nonpeptidergic neuromodulators, except the classic neurotransmiters (noradrenalin, acetylcholin) The nitric oxide (NO) has been shown to be one of the neurotransmitters of the peripheral nervous system, which takes part in nonadrenergic and noncholinergic neurotransmission (Grozdanovic *et al* 1992, 1994) The NADPH-diaphorase is an enzyme that shows specific sites of the NO (Hope *et al* 1991) As is suggested, NO may participate in the regulation of spleen functions together with other neurotransmitters The present report analyzes the degree of colocalization of both enzymatic markers in neuronal structures of the phaesant spleen, carried out by successive ACHE and NADPH-d histochemistry in the identical sections

Male and female phaesants were anesthetized with pentobarbital in the period of 1–5 weeks after the birth Animals were perfused intracardially by Ringer solution followed by fixative solution containing 4% paraformaldehyde and 0,1% glutaraldehyde in 0 1 mol/l phosphate buffer (PB), pH 7 4 The spleen was dissected, postfixed in the same fixative

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Figure 1. The pheasant spleen – thick and fine ACHE-positive nerve fibres (arrows) enter the hilum in the vicinity of the splenic artery branches Abundand occurrence of ACHE-positive nerve fibres in the red pulp, was observed as well

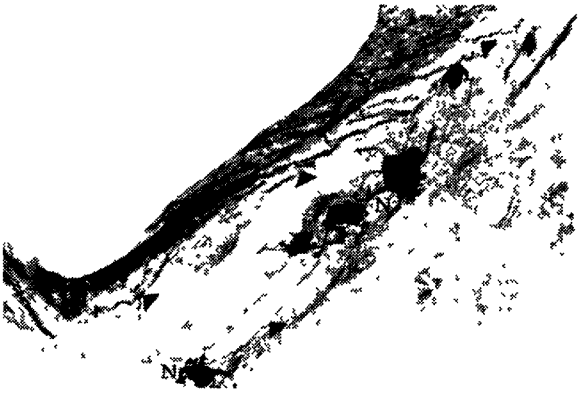

Figure 2. The pheasant spleen – amounts of fine NADPH-d positive nerve cells (N) and NADPH-d positive nerve fibres (arrow heads) in periarterial localization

solution for 2 hours and then stored for overnight in 30% sucrose in 0 1 mol/l PB Twenty-micrometer sections were cut using a freezing microtome Histochemical procedures were carried out on serial free-floating sections One-in-three series from each animal was processed for demonstration of NADPH-d activity described by Scherer-Singler *et al* (1983) The second set was used for ACHE histochemistry by direct tiocholine method described by El-Badawi and Schenk (1967) and the third set was processed for both enzymatic activities at the same sections (Crespo *et al* 1995) The ACHE histochemistry was carried out in the sections previously processed for NADPH-diaphorase Sections were mounted on slides and coverslipped with Entellan

Two types of nerve structures were found in the pheasant spleen nerve fibres and nerve cells Both enzymatic activities were present in the nerve fibres NADPH-d and ACHE-positive nerve fibres enter the spleen in its hilum in the vicinity of the splenic artery branches (Fig 1,2) Both visualized nerve fibres were gradually distributed in periarterial topography in the white pulp of the spleen In the red pulp, only ACHE-positive nerve fibres were found (Fig 1) Althouth both enzymatic activities were present in periarterial nerve fibres, colocalization of NADPH – diaphorase and acetylcholinesterase was not observed in the nerve cells NADPH-d positive nerve cells have been seen around

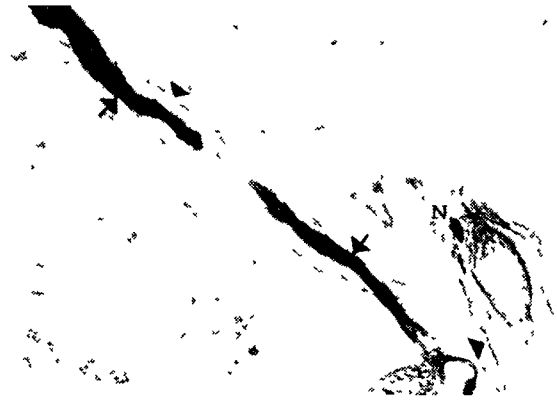

Figure 3. The pheasant spleen – NADPH-d (arrows) and ACHE – (arrow-heads) positive nerve fibres are visible in their periarterial colocalization Only NADPH-d positive nerve cells (N) are without the ACHE-positivity

the splenic vessels especially at the hilum of the spleen They were polygonal in the shape and occured as solitary or numerous cells, forming ganglia (Fig 3) No NADPH-d positive nerve fibres or nerve cells were found in the splenic capsule Only fine ACHE-positive nerve fibres were present there

Our findings suggest the coexistence of the nerve fibres positive for acetylcholinesterase and NADPH-diaphorase in the white pulp of the pheasant spleen in their periarterial course Colocalization of the NADPH-d positive nerves with nerves expressing ACHE in perivascular topography suggests that NO may play the role as a messenger in the functions of the splenic vessels either directly or through the interaction with other neurotransmitters

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