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TYPES OF NEURONES REVEALED BY NBT/NADH STAINING IN THE ENS OF STURGEON AND CARP

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Abstract

A chondrostean and a teleostean fish: sturgeon (*Acipenser ruthenus*) and carp (*Cyprinus carpio*) were studied for the comparison of their enteric nervous system, especially the distribution and size of myenteric neuron types with light microscopic NADH-diaphorase staining.

Although the gross morphology of the sturgeon alimentary tract was found to be more complicated than that of the carp, the histological layers of the various gut segments were similar in both species. The NBT/NADH staining revealed both neurons and nerves in the myenteric plexus of sturgeon, while only perikarya were stained in the carp. The number of stained cells was obviously less in the sturgeon but their size was considerably larger than that of the neurons in the carp myenteric plexus. Three types of stained myenteric neurons were described in both sturgeon and carp: large bi- and multipolar types, moreover a small cell type with narrow cytoplasmic rim around the fairly large nucleus. The average diameter of large cells was measured around 50-60 μm in the sturgeon and about 15 μm in the carp. The small cell type had an average diameter of 20 μm in the sturgeon and less than 10 μm in the carp.

The large multipolar cells are thought to be intrinsic sensory and motoneurons, the bipolar cells can be considered as interneurons and the small neurons as undifferentiated stem cells.

Key words: fish, enteric neurons, morphology, NBT/NADH

Introduction

With the recalling of LANGLEY's (1921) concept of enteric nervous system (ENS) neuroscientists hoped to get access to a model-system which seemed to be less complicated than the central nervous system (CNS). The ENS is easily accessible even in the living animal and its main, well-defined function is the regulation of the gut peristalsis (GERSHON, 1981). The main subject of these studies has been the mammalian ENS (GABELLA, 1972; FURNESS and COSTA, 1980; GERSHON, 1981; BURNSTOCK, 1986). However its complexity became soon evident and the interest turned the ENS of lower vertebrates, especially some groups of economic importance like fish and birds (BURNSTOCK, 1959; SALIMOVA and FEHÉR, 1981; ANDERSON, 1983; ROMBOUT and REINECKE, 1984; HALASY and BENEDECZKY, 1985; HOLMGREN, 1985; BUDDINGTON, 1986; HALASY et al., 1986;

ROMBOUT et al., 1986; BJENNING and HOLMGREN, 1988; BURKHARDT-HOLM and HOLMGREN, 1989). Amazing differences were found between the various fish species according to the organization of their ENS (HOLMGREN, 1985). Our aim was in this study to reveal some morphological similarities and differences between the ENS of two different fish species belonging to different subclasses.

Materials and methods

The adult individuals of sturgeon (*Acipenser ruthenus* L.) and carp (*Cyprinus carpio* L.) were obtained from the Szarvas Fish Breeding Research Institute.

The fish were killed by a blow on their heads, the alimentary tract was removed and distended in Krebs-solution in the form of small sausages. These were kept for 15 minutes in Krebs solution containing 0.3 % Triton X 100, then incubated in NBT/NADH solution for 30 minutes. (For details of the method see: *Gabella*, 1967). After the incubation the material was fixed in 10% neutral formalin for at least 24 hours, then 0.5-1 cm rings were cut from the gut wall, the mucosa was removed and the remaining muscular layers were stretched on slides as whole mounts in glycerol and covered with cover slips. Photographs were taken in a Zeiss light microscope.

Results

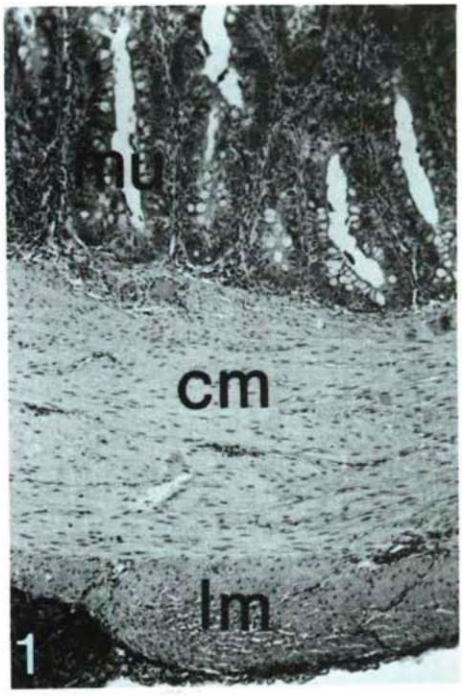
Comparing the gross morphology of the alimentary tract in the two studied species, the gut of the chondrostean sturgeon was found to be more complicated, than the teleostean carp's one. While the carp gut is a fairly simple tube with various diameters and wall thickness in the different gut segments, the sturgeon gut has clearly distinguishable regions, namely: the foregut consisting of the esophagus, proventriculus and ventriculus with the pyloric appendices; the midgut with the hepatopancreas on its outer surface and the spiral valve; then the last segment is the relatively short hindgut.

Fig. 1: Cross section of the midgut of sturgeon. mu = Mucosa; cm = circular smooth muscle layer; lm = longitudinal smooth muscle layer. Haematein-eosin staining. x100.

Fig. 2: Cross section of the carp midgut. sm = submucosa; cm = circular smooth muscle layer; lm = longitudinal smooth muscle layer. Haematein-eosin staining. x200.

Fig. 3: A nerve bundle (asterisks) between the circular (cm) and longitudinal (lm) muscular layers in the midgut of sturgeon. Haematein-eosin staining. x400.

Fig. 4: A germinal center (arrow) in the lymphoreticular tissue of spiral valve of sturgeon gut. Haematein-eosin staining. x150.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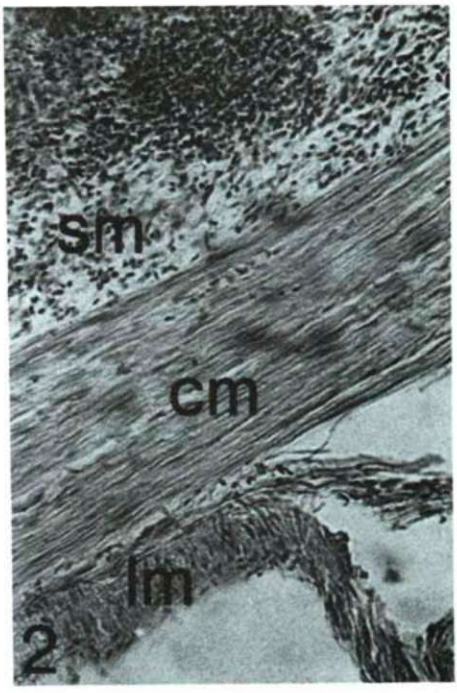
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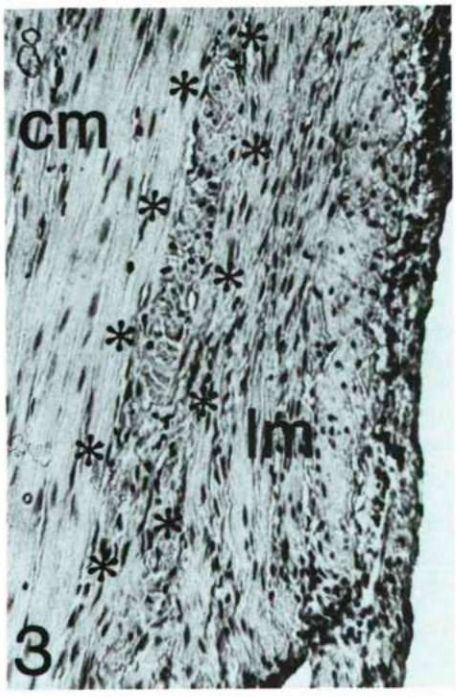
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In spite of the conspicuous anatomical differences the histological layers of the gut wall are very similar in the two studied species (Figs. 1, 2) corresponding to the conventional layering of hollow visceral organs (mucosa, submucosa, muscularis and serosa). As a histological speciality, the axis of the spiral valve in the sturgeon was found to contain lymphoreticular tissue with germinal centers (Fig. 4). Our studies were focussed on the myenteric plexus which lies between the inner circular and outer longitudinal smooth muscular layers muscularis (Fig. 3).

The NADH-diaphorase staining revealed perikarya and network of nerves in the sturgeon gut (Figs. 5-9). A number of thick nerves running longitudinally and parallelly with each other were heavily stained (Fig. 5). At places, besides the parallelly running nerves, irregular network of nerves was visualized between the two muscular layers (Fig.6). Stained perikarya were found along the entire gut evenly but sparsely distributed (Figs. 6-9). They did not form conventional ganglia but sometimes the various cell types gathered into little groups (Fig. 7). Three types of perikarya could be distinguished according to their morphology and size: 40-60 μm large multipolar (Figs. 7-9), 60 μm elongated bipolar (Fig. 7), and cca 20 μm small cells with narrow cytoplasmic rim around the relatively large nucleus (Fig. 7). The staining was confined to the cytoplasm and at a higher magnification its grainy character became well visible (Fig. 9).

Considerably higher number of perikarya and no nerves were stained in the carp myenteric plexus along the entire length of the alimentary tract (Figs. 10-13). The cells were scattered between the circular and longitudinal muscular layers, several of them were solitary (Figs. 11.13), but small ganglion-like groups of few cells were here also frequent (Fig. 12). The considerably higher density of neurons was combined with smaller size: here the average diameter of larger multi- and bipolar cells was measured around 15 μm (Figs. 11-13), while the average diameter of the smaller cells somewhat below 10 μm (Figs. 10. 12). Sometimes the processes of the neurons were also stained especially those of the bipolar cells (Figs. 11. 13).

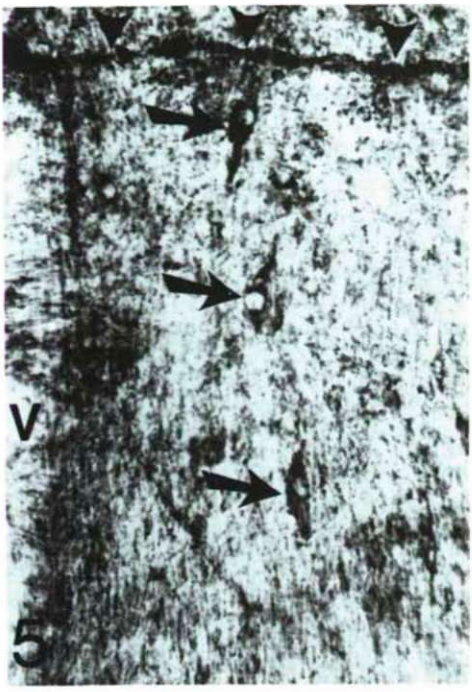
Fig. 5: A row of bipolar neurons (arrows) arranged parallelly with a blood vessel (v) in the sturgeon hindgut. Note the stained nerve (arrowheads, on the top of the picture. NBT/NADH staining, whole-mount preparate. x200.

Fig. 6: Network of stained nerves with a perikaryon (arrow) at the junction of nerves in the sturgeon hindgut. NBT/NADH staining, whole-mount preparate. x150.

Fig. 7: Ganglion-like arrangement of large (arrows) and small (open arrows) perikarya in the muscular layer of sturgeon hindgut. NBT/NADH staining, whole-mount preparate. x40.

Fig. 8: Large multipolar neuron (arrow) in the sturgeon hindgut. NBT/NADH staining, whole-mount preparate. x450.

Fig. 9: High magnification of a multipolar myenteric neuron with two stained process (arrows). Note the granular appearance of the cytoplasmic staining and the lack of staining over the nucleus. NBT/NADH staining, whole-mount preparate. x800.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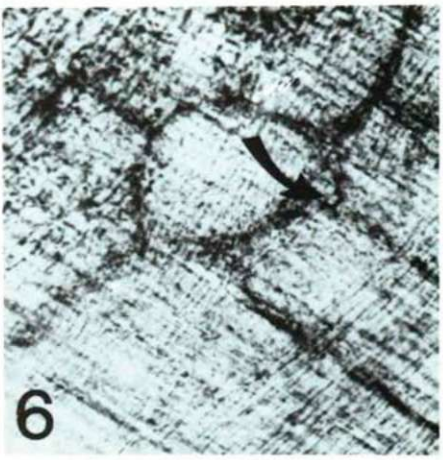
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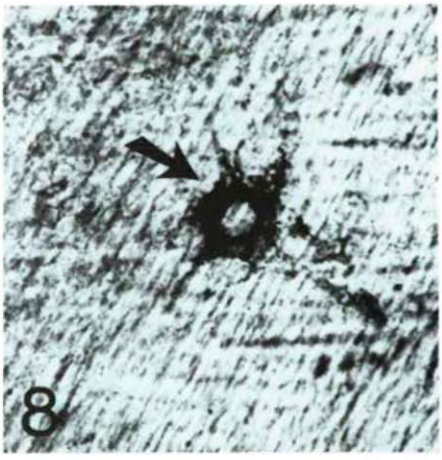
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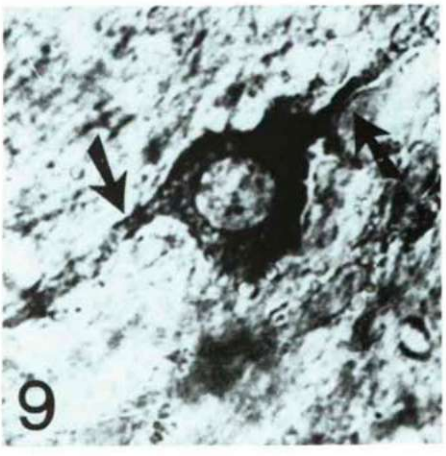
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Discussion

Previous observations (WATSON, 1981) established that the organization of the fish ENS is less complicated than the mammalian one. However it is fairly difficult to draw general conclusions valid for a whole taxonomic group. This is true especially in the case of fish because this group contains cca 25.000 species (HOLMGREN, 1985). This fact also emphasizes the importance of comparative studies even inside one taxonomic group. For this reason we have chosen two fish species belonging to different subclasses for the comparative morphological study of their ENS.

For this purpose the NADH-diaphorase method – staining first of all neuronal perikarya – proved to be very suitable. In previous studies (GABELLA, 1987; HALASY et al., 1988; GÁBRIEL et al., 1988; 1989) this method was successfully used for the description of the general pattern of myenteric plexus and for the study of distribution of neurons in different species, and even for the counting of the neuron-number in various segments of the gut.

The most contradictory data are available on the question of ganglionic arrangement of myenteric plexus in the fish gut. KIRTISINGHE (1940) studying the teleostean indian catfish (*Sacchobranchus*) did not find ganglia, but solitary neurons in the ENS. BURNSTOCK (1959) found ganglionlike groups of nerve cell bodies at the nodal points of nerves in the ENS of brown trout (*Salmo trutta*). BJENNING and HOLMGREN (1988) studying the occurrence of neuropeptides in the fish gut, made experiments on 33 fish species. They established that there are strong specific differences in the organization of their ENS. GÁBRIEL et al. (1988) published two steps of the phylogenetic development of ganglionated plexus: only the foregut was found to have ganglionated myenteric plexus in the frog and lizard. The well-developed ganglionated myenteric plexus appeared first in the entire alimentary tract of birds during the phylogenesis.

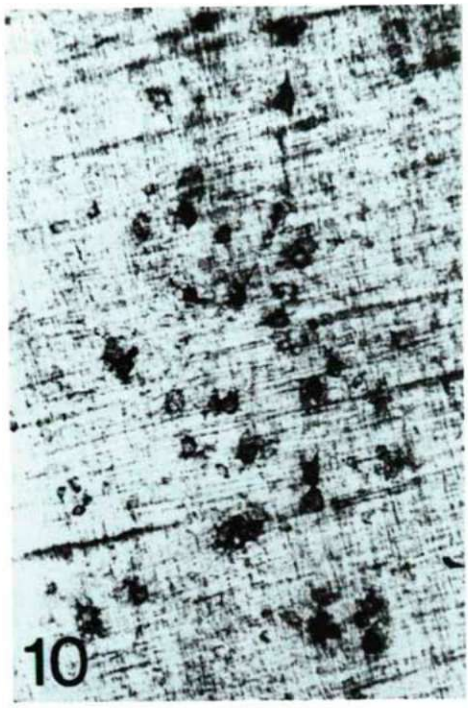
Neither the sturgeon nor the carp ENS showed ganglionic arrangement in our study. The appearance of small nerve cell groups in both species can be evaluated as a preliminary step to a ganglionated system.

Fig. 10: Characteristic pattern of the distribution of myenteric neurons in the midgut of carp. NBT/NADH staining, whole-mount preparate. x200.

Fig. 11: A bipolar (arrow) and a multipolar neuron (open arrow) between the muscular layers of carp midgut. NBT/NADH staining, whole-mount preparate. x400.

Fig. 12: Ganglion-like group of large (arrow) and small (arrowheads) perikarya in the midgut of carp. Note the very narrow cytoplasmic rim around the nucleus of small type cells. NBT/NADH staining, whole-mount preparate. x800.

Fig. 13: A solitary, elongated bipolar neuron in the midgut musculature of carp. NBT/NADH staining, whole-mount preparate. x900.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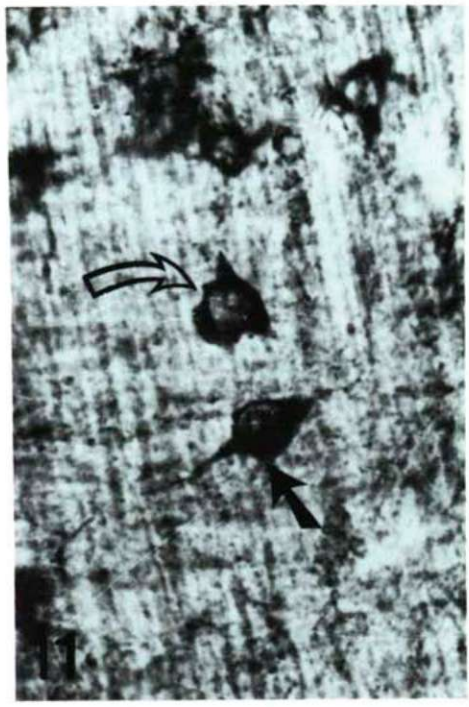
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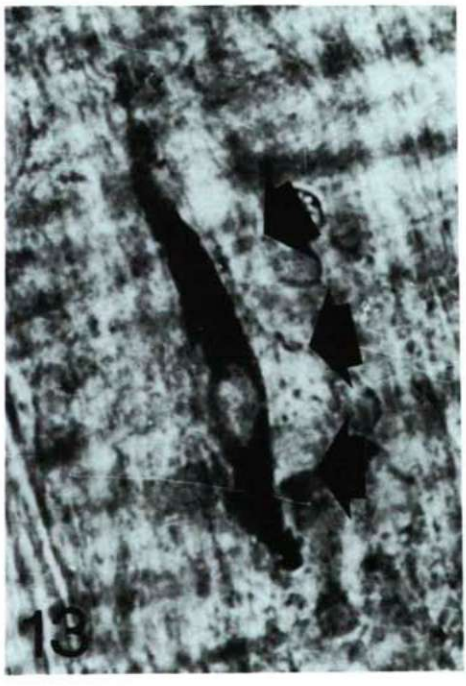
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Data received with the application of a purely morphological method are not satisfactory to elucidate the function of the intrinsic neurons present in the ENS. Among the multipolar cells very probably both sensory and intrinsic motoneurons are present, while the bipolar types can be associative or interneurons (BURNSTOCK, 1959). The function of the small neurons is even more questionable. Studying the neuron size and number in the rat myenteric plexus GABELLA (1971) raises the possibility that undifferentiated neuroblasts are present even in the adult myenteric plexus and these immature nerve cells are able to differentiate to neurons when required. The small cell-types in the studied fish ENS may represent a pool of immature nerve cells too, and this is the morphological basis of neuronal plasticity characteristic of all parts of the nervous system.

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