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COMPARATIVE MORPHOLOGY OF THE NEUROMUSCULAR JUNCTIONS IN THE ALIMENTARY TRACT OF SOME INVERTEBRATE AND VERTEBRATE ANIMALS

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Introduction

The concept of the distinction of the enteric nervous system (ENS) as a third division of the autonomic nervous system was raised by LANGLEY (1921). He first realized the specific autonomic function of visceral organs merely modified by the sympathetic and parasympathetic influence. He suggested the separation of the ENS from the sympathetic and parasympathetic nervous system on the basis of:

- its relative independence of the central nervous system
- the presence of complete reflex arches in the gut wall
- the extremely high number and diversity of enteric neurons.

Recent fine structural studies (GABELLA, 1972; 1981; FURNESS and COSTA, 1980; GERSHON, 1981; BURNSTOCK, 1986) showed that the ultrastructure of the ENS resembles more that of the central nervous system than the other parts of the peripheral nervous system.

Cytochemical and pharmacological studies proved that the intramural neurons of the alimentary tract contain a large variety of neurotransmitters and/or neuromodulators just like the neurons of the central nervous system.

Since experiments have been carried out mostly on mammals, we decided to study the ENS of some lower invertebrate and vertebrate species for a comparative morphological analysis. Besides an overview of snail, insect and fish ENS, a special attention was paid to the fine structure of the enteric neuromuscular junctions playing a key role in the motility of the gastrointestinal tract.

Our questions were:

- What forms of neuromuscular junctions are to be found in the enteric muscular layers of the studied invertebrate and vertebrate species?
- What kind of vesicle populations are characteristic of the axon profiles taking part in the enteric neuromuscular junctions?
- What are the neurotransmitters and/or neuromodulators present in those axon profiles?
- How does the transmitter release take place?

– What conclusion can be drawn from the comparison of our results with those received on mammals?

Materials and methods

Adult specimens of snail (*Helix pomatia*), locust (*Locusta migratoria*), cockroach (*Periplaneta americana*), sturgeon (*Acipenser ruthenus*), carp (*Cyprinus carpio*) and tench (*Tinca tinca*) were used.

The applied methods were:

1. *Light microscopic embedding and staining*: after fixation in 4 % formaldehyde or Bouin fixative tissue blocks were embedded in paraffin, 7-10μm sections cut and stained with haematein and eosin.
2. *NADH-diaphorase method*: for the visualization of nerve elements gut segments distended with Krebs solution, treated with Triton X-100 were incubated in a reaction mixture containing NBT and NADH (GABELLA, 1967). Gut segments were then fixed in 10 % neutral formalin, whole mount preparates of the muscular layers made, and mounted on slides in glycerine.
3. *Light microscopic localization of AChE*: a modified version of KOELLE and FRIEDENWALD (1949) method was applied on whole mount preparates.
4. *Light microscopic immunocytochemistry*: STERNBERGER's preembedding immunoperoxydase method was applied to whole mounts for the localization of proctolin, serotonin, dopamine (antisera kindly donated by MANFRED ECKERT), GABA, and FMRFamide (antisera kindly donated by PETER SOMOGYI).
5. *Electron microscopy*: for the study of the fine structure of the enteric neuromuscular junction tissue blocks were immersed in, or perfused with 3 % glutaraldehyde, or Karnovsky-fixative, embedded in Durcupan ACM, double-stained with uranyl acetate and lead citrate. Ultrathin sections were cut and studied in JEOL 100 C, or Tesla BS 500 electron microscope.
6. *Electron microscopic detection of AChE*: the method described by TÓTH (1977) was applied for the detection of cholinergic motor endplates in the striated musculature of fish alimentary tract.
7. *TARI method*: for the visualization of exocytosis profiles of non-synaptic transmitter release tissue blocks were incubated in a medium containing tannic acid (BUMA et al., 1984), then treated as described in point 5.
8. QUANTITATIVE DETERMINATION OF BIOGENIC MONOAMINES AND AChE: The amount of adrenaline and noradrenaline was determined by ANTON and SAYRE (1962), the amount of dopamine by SCHELLENBERG and GORDON (1971), the 5-HT was determined with the fluorimetric method of SNYDER et al. (1965). The AChE-activity was measured by ELLMAN et al. (1961).

Results

The innervation of the gut musculature of *Helix pomatia* morphologically resembles the mammalian myenteric plexus in several respect. The alimentary tract has a smooth muscular layer rich in nerve elements and intrinsic neurons. The neuromuscular junctions are similar to the mammalian autonomic close contacts. Three types of axon terminals (T1: containing 200 nm dense ellipsoidal granules; T2: containing 100 nm dense-core vesicles; T3: containing 250 nm granules with grainy matrix) establish close contacts with the smooth muscle cells in the gut wall of snail. Besides the similarities essential differences were revealed: in the snail ENS no

synapses are present in the neuropil; the majority of the myenteric neurons are unipolar; the neurons do not form ganglia; the number of morphologically distinguishable axon profiles is lower than in the mammalian ENS. With the help of TARI-method we could prove that non-synaptic transmitter release takes place both in the neuropil and myoneural close contacts. Exocytotic transmitter release was detectable even if the distance between the nerve and muscle was considerable.

Anthropods representing a different phylogenetic line have a different way of innervation in their alimentary tract. The exclusively cross-striated enteric musculature receives both synaptic and non-synaptic (close contact) innervation. Two, morphologically different axon terminals establish synaptic neuromuscular junctions (T1: contains 50 nm agranular vesicles together with 130 nm granules; T2: 50 nm agranular vesicles are in it together with 200 nm moderately electron dense granules). Four types of axon terminals with very diverse vesicle population establish close contacts with the striated muscles (T3: containing 130 nm electron dense granules; T4: with 150 nm strongly electron dense granules; T5: 150 nm strongly electron dense granules and 50 nm agranular vesicles; T6: 120 nm dense-core vesicles). Intramural neurons were found in the hindgut of locust by means of NADH-diaphorase method. Their sensory or motor character has not been proved yet.

The alimentary tract of the studied fish species – representing the beginning of the vertebrate evolution – is. built up by similar histological layers like that of mammals. The tunica muscularis is built up by circular and longitudinal smooth muscle layers. In the tench striated muscular layers are attached to the smooth smooth ones till the border of midgut and hindgut. This is a peculiarity restriced to this species, its physiological role has not been clarified yet. Both types of muscular layers are innervated by the same nerve. plexus. The majority of the myenteric neurons are multipolar. They do not gather into ganglia in the studied fish species. The nerve-smooth muscle junction is the autonomic close contact, the nerve-striated muscle contact is the motor endplate. Its morphology differs from that of mammalian motor endplates: the finger-like infoldings of sarcolemma are not present. Morphologically three different types of varicosities are to be found both in the myenteric plexus and nerve-muscle junctions (T1: 40-50 nm pleomorph agranular vesicles and 100-120 nm dense-core vesicles; T2: 200 nm dense granules; T3: 40-50 nm agranular vesicles).

Biochemical measurements proved the presence of norepinephrine, epinephrine, dopamine and serotonin in the gut of each studied group. In the snail and carp serotonin was found to be present in highest amount, in the locust the amount of dopamine was the highest.

Serotonin and dopamine was detected by immunocytochemistry in the snail gut, both in the nerves and intrinsic perikarya. Nerve fibres showing FMRFamide-like immunoreactivity were also present in the snail gut. Varicose nerve fibres of locust hindgut showed serotonin-like and proctolin-like immunoreactivity. A GABA-

ergic groundplexus was revealed in the carp gut by immunocytochemistry. The presence of AChE was also detected in nerves and intramular nerve cell bodies in different regions of snail and fish gut. The activity of AChE was measured significantly higher in the tench fore- and midgut, than in the hindgut, or any gut segments of the carp. The higher enzyme activity can be related to the presence of striated muscular layers.

Summarizing our results, we contributed to the understanding of the evolution of the ENS with some new morphological data. Our results provide structural basis for further functional experiments.

References

ANTON, A.H. and SAYRE, D.F.(1962): A study of factors affecting the aluminium-oxyde-trihydroxyindole procedure for analysis of catecholamines.-J. Pharmac.exp.Ther. *138*, 360-372.

BUMA, P., ROUBOS E.W. and BUIJS, R.M.(1984): Ultrastructural demonstration of exocytosis of neural, neuroendocrine and endocrine secretion with an in vitro tannic acid (TARI) method.-Histochemistry *80*, 247-257.

BURNSTOCK, G.(1986): Autonomic neuromuscular junctions: current developments and future directions.-J. Anat. *146*, 1-30.

ELLMAN, G.L., COURTNEY, D.D., ANDRES V. and FEATHERSTONE, R.M.(1961): A new and rapid colorimetric determination of acetylcholinesterase activity.-Biochem. Pharmacol. *7*, 88-95.

FURNESS, J.B. and COSTA, M.(1980): Types of nerves in the enteric nervous system.-Neuroscience *5*, 1-20.

GABELLA, G.(1967): Detection of nerve cells by a histochemical technique.-Experientia *25*, 218-219.

GABELLA, G.(1972): Fine structure of the myenteric plexus in the guinea-pig ileum.-J. Anat. *111*, 69-97.

GABELLA, G.(1981): Ultrastructure of the nerve plexuses of the mammalian intestine: the enteric glial cells.-Neuroscience *6*, 425-436.

GERSHON, M.D.(1981): The enteric nervous system.-Ann. Rev. Neurosci. *4*, 227-272.

KOELLE, G.B. and FRIEDENWALD, J.S.(1949): A histochemical method for localizing cholinesterase activity.-Proc. Soc. exp. Biol. (N.Y.) *70*, 617-622.

LANGLEY, J.N.(1921): The autonomic nervous system. Part.I. Cambridge: W. Heffer.

SCHELLENBERGER, M.K. and GORDON, J.H.(1971): A rapid simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytriptamine from discrete brain areas.-Anal.Biochem. *39*, 356-372.

SNYDER, S.H., AXELROD, J. and ZWEIG, M.(1965): A sensitive and specific fluorescence assay for tissue serotonin.-Biochem. Pharmac.*14*, 831-835.

TÓTH, L.(1977): Biodynamics of acetylcholinesterase in the motoneural unit. C. Sc. Dissertation, Szeged.