NITRIC OXIDE CONTAINING NERVE ELEMENTS
IN THE GASTROINTESTINAL TRACT

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**Introduction**

The descending inhibitory innervation of the gastrointestinal tract occurs mainly via non-adrenergic, non-cholinergic (NANC) nerves. The possible mediators of these nerves are the vasoactive intestinal polypeptide (VIP), adenosine-triphosphate (ATP) and also *non-purinergic, non-peptidergic* neurotransmitters like nitric oxide (NO).

Besides the intramural, enteral neural elements, the *interstitial cells of Cajal* have also important regulatory role in the coordination of normal enteral motility.

**Objectives**

1) To study the **distribution** and the **localization** of the nitric oxide containing nerve elements (neurons, nerve fibres) in the gastrointestinal tract.

   a) To localize nitrergic nerve elements in different regions of the alimentary tract.

   b) To localize nitrergic nerve elements in the different layers and structures of the wall of specific enteral regions.

   c) According to the literature, the presence of nicotinamide-adenine-dinucleotide-phosphate-diaphorase (NADPH-d) enzyme can be the marker of neural nitric oxide synthase (nNOS) activity in the peripheral nervous system, therefore we compared these enzyme- and immunohistochemical methods in the neural elements of the gastrointestinal tract.

   d) To compare the distribution and localization of the nitrergic nerve elements in *sphincteric* and *non-sphincteric regions*.

2) To describe the distribution and localization of the nitrergic and VIP containing nerve elements in the *pylorus* and compare the localization of these transmitters.
3) To identify the possible target cells (smooth muscle cells, glandular epithelium, surface epithelium, other neurons and cellular elements of the immune system) of NO containing nerve elements in the pylorus of the cat, using light and electone microscopy.

4) To analyse the localization of nNOS in the colon during experimental, induced colitis ulcerosa and study the possible changes of the interstitial cells of Cajal during experimental colitis.

**Materials and methods**

**Animal experimental** samples were taken from:

- Cat: pylorus, ampulla of the hepatopancreatic duct (sphincter of Oddi), ileum, ileocecal junction, colon.
- Rat: colon.

**Experimental methods:**

- **NADPH-d enzymehistochemistry, nNOS immunohistochemistry.**
- **nNOS immunocytochemistry**: sections were cut by Vibratome. Immune reaction, postfixation with osmium tetroxide then embedding. Ultrathin sections (45-80 nm) were made, then stained with uranyl-acetate and lead citrate. Examination under Jeol 100 electrone microscope.
- **Experimental trinitrobenzene sulphonic acid (TNBS)-induced colitis.**

TNBS was injected intraluminally into the descending colon of the rats, where it caused a moderate colitis in all the animals. nNOS and VIP immunohisto- and immunocytochemistry were performed on sections from inflamed and non-inflamed area of the colon 1 and 2 days after the TNBS treatment.
All experimental procedures used conformed to the “Principles of laboratory animal care” (NIH publication No. 86–23, revised 1985) and specific Hungarian national laws as well (No. 243/1998).

**Quantitative analysis:** the positive neurons and nerve fibres were examined in a 2000-3000 \( \text{mm}^2 \) tissue area and their number was counted for 100 \( \text{mm}^2 \) area.

**Results**

**Examination of different regions of the alimentary tract - especially of the pylorus**

NADPH-d and NOS positive *neural cell bodies* were found in all the studied regions of the gastrointestinal tract, especially in the *myenteric plexuses*. Some multipolar neurons were seen in the submucous plexus and rarely in the inner, circular smooth muscle layer and in the tunica mucosa as well.

NO containing *nerve fibres* were seen in similar distribution and localization using NADPH-d enzyme- and NOS immunohistochemical methods. The highest number of nitrergic nerves were seen in the inner, circular muscle layer and none of them or very few were found in the outer, longitudinal muscle layer. Positive fibres were also found in the submucosa, close to the blood vessels and in the tunica mucosa around the glands or beneath the epithelium.

The nitrergic neural cells were found in significantly higher amount in the myenteric plexuses of the *sphincter regions* (pylorus, sphincter of Oddi, ileocecal junction).

The distribution and localization of NOS and VIP immunoreactive (IR) nerve elements was similar in the pylorus.

Immunocytochemistry demonstrated NOS IR nerve terminals containing numerous large granular (diameter: 80-120 nm) and small clear (diameter: 30-
40 nm) vesicles. NOS IR nerve fibres were demonstrated forming *interneuronal synapses* in both intramuralplexuses with immunonegative neurons.

Large numbers of NOS IR nerve terminals were found in close vicinity to the smooth muscle cells of the inner, circular muscle layer. Nitrergic nerve fibres were also seen in the close vicinity of the capillary endothelium and the vascular smooth muscle cells. In the tunica mucosa NOS IR nerve terminals were found in close situation to immunocompetent cells (lymphocytes, plasma cells). The gap between the NOS IR nerve fibres and the membrane of the possible target cells was 20–250 nm. (According to the generally accepted opinion, in case of distant synapses the maximal width of the synaptic gap can be 1µm in order to have physiologic effects.)

**In experimental colitis:**

1 day after the TNBS treatment mild inflammation, 2 days after the treatment moderate colitis was seen. The myenteric and submucous plexuses as well as the tunica muscularis seemingly were intact.

In the inflamed area the numbers of NOS and VIP IR nerve fibres were slightly decreased, however, large numbers (24%) of the *interstitial cells* became IR for NOS. These cells were located in the inner part of the circular smooth muscle layer (*submucosal plexus*). In the non-inflamed area and in the control samples these cells remained unlabeled.

The NOS IR nerves were closely situated to the Cajal cells. The IR Cajal cells exhibited close contacts with the smooth muscle cells also. The gap between the IR Cajal cells and the membrane of smooth muscle cells was 20–30 nm.
Discussion

NO containing neurons of the enteral system are mainly situated in the myenteric plexuses. Earlier studies described NADPH-d positive neurons only in the myenteric plexus. These neurons can regulate the aboral smooth muscle relaxation in the peristaltic reflex. We have also demonstrated nitrergic neurons in the submucous plexus which can influence the gastrointestinal blood flow or the secretion.

NOS IR nerve fibres form synapses in both intramural plexuses, suggesting the postynaptic effect of NO on these neurons, thus participating in the local neuronal reflexes.

NO containing nerve elements can be found in higher density in the sphincter regions of the gastrointestinal tract (pylorus, sphincter of Oddi, ileocecal junction). This distributional pattern suggests the significant inhibitory innervation in these regions.

The marked decrease of the nitrergic nerve fibres in experimental colitis can be due to the neural degeneration during the acute inflammation or – without degeneration- the NOS synthesis can be downregulated in these neurons.

Our light- and ultrastructural studies suggest that some of the interstitial cells of Cajal can synthesize NO, at least in certain conditions (e.g. during inflammation). The NO probably produced by these cells can substitute the decrease of neurally released NO. The change in the number and structure of the interstitial cells can play an important role in the pathogenesis of a variety of motility disorders.
Conclusions

The following new observations were made in our investigations:

- The distribution and density of the nitrergic nerve elements are different in the gastrointestinal tract but greater numbers of NO containing nerve elements are found in the myenteric plexuses and the inner, circular smooth muscle layer of the sphincter-regions. This distribution pattern suggests that nitrergic nerves cause smooth muscle relaxation in the sphincter regions.

- In the pylorus we have demonstrated NOS immunoreactive nerve terminals in both intramural nerve plexuses forming intraneuronal synapses. These results suggest the postsynaptic effect of NO on these plexus neurons and thus participation in the local neural reflexes.

- The similar distributional and localizational patterns of VIP and NOS immunoreactive nerve elements suggest the possible colocalization of these transmitters in the inhibitory neurons of the pylorus.

- We have observed nitrergic nerve terminals in close apposition to the vascular smooth muscle cells, suggesting the regulating effect of NO in the blood flow and thus in the gastroprotection. NO containing nerve terminals were also found around the pyloric glands and beneath the surface epithelium suggesting the nitrergic modulation of gastric secretion.

- In the experimental colitis model we have demonstrated the marked decrease of the number of NOS and VIP containing nerve terminals and their synapses during moderate inflammation. We found that some of the interstitial cells of Cajal in the inner, circular smooth muscle layer of the inflamed colon became NOS immunoreactive. This change can modulate the motility during inflammation and suggests innervation plasticity of the enteral system.
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Publications

**Original articles related to the thesis project:**

**Abstracts related to the theses**

**Posters and lectures**


5. Ember Zs, **Altdorfer K.** A nitrogén-monoxid kimutatásának morfológiai alapjai. XXVIII. Membrán-transzport Konferencia, Sümeg 1998.


**Articles not related to the thesis**
