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Distribution of nitric oxide synthase in stomach wall in rats

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INTRODUCTION

It has been shown that neuronal nicotinamide adenene dinucleotide phosphate-diaphorase (NADPH-d) may correspond to the neuronal nitric oxide synthase (NOS), and may be used as a marker for NOS in the central and peripheral nervous system. Thus, NADPH-d histochemistry provides us with a mean to specifically identify neurons producing nitric oxide (NO)[1,2].

Recent pharmacological and physiological-studies demonstrated that NO is a neurotransmitter in the non-adrenergic non-cholinergic (NANC) inhibitory nerves in the mammalian gastrointestinal tract. It may play a very important role in then euronal regulation of gut. NOS activity is present in neurons and fibers of the major enteric nerve layer in intestine[3]. However, there have been far fewer studies of NOS activity in stomach wall. If NO is a transmitter of NANC in hibitory nerves, it should be present in neurons innervating the muscularis. What proportion of nerves produce NO? What is the pattern of innervation of these neurons? To answer these questions, we examined the distribution and morphological feature of NOS positive neurons in the stomach wall with improved whole mount preparation technique.

MATERIALS AND METHODS

Adult male Wistar rats weighing 210g-250g were provided by the Center of Laboratory Animals of our university. The experimental rats were fasted overnight prior to the experiment, and anaesthetized with sodium pentobarbitone (50 mg·kg^{1}, ip). Stomach was excised and rinsed with PBS and dipped in 40 mL/L paraformaldehyde for 4 h, then stored in 200 g/L sucr ose in PBS for 48 h at 4℃. Thereafter, whole mount preparations were made, and histochemistry staining of NADPH-d and control test were performed as previously reported[4].

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Statistical analysis

All results were expressed as the $\bar{x}\pm s$, and data were analyzed by S tudent's t test. P values <0.05 were considered statistically signifi cant.

RESULTS

Our study showed that NOS was widely distributed in the gastric wall, and most of them were located in the myenteric plexus, and distributed in submucosal plexus, gastric mucosal epithelium and gastric gland. In the myenteric plexus, the cytoplasm of the NOS positive neurons completely labelled except for the nucleus. The cell body shape was basically similar, most of them shaped round, oval or fusiform while their density, size and staining intensity varied greatly in the different parts of stomach. The density was (62 ± 38) cells/mm^2, (43 ± 32) cells/mm^2 and (32 ± 28) cells/mm^2 respectively in the antrum, body and fundus (P<0.01). Tow subtypes of NOS positive neurons could be distinguished on the basis of size, staining intensity and number of progresses. In fundus, about 75% neurons were large, and dark-stained. Neurons of the second subtype were sligh tly smaller, with only one or two processes and were mainly located in the antrum (approximately 65%). In the body of stomach, the character of NOS positive neu rons was an intermediate state from fundus to antrum.

DISCUSSION

The results of our experiment provide the first morphological evidence for the presence of NOS positive neurons in the stomach myenteric plexus. Nerve bundles also contained a large number of reactive fibers. Many bead-like structures strung together by NOS positive varicosities in nerve fibers, some were closely adherent to the outer walls of blood vessels and smooth muscle fibers. This finding has provided morphological evidence of NO involved in the modulation of motility and blood circulation of gastrointestinal tract. The significant difference of the distribution of NOS positive neurons among the myenteric plexus in different parts of the stomach may be related to the physiological function of the stomach.

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