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Innervation of the Muscle Coat of the Recto-Anal Region in the Rat

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The distribution of nerve cell bodies and fibres which contain adenosine triphosphate (ATP), nicotine amide adenine dinucleotide phosphate-diaphorase (NADPH-d), choline acetyltransferase (ChAT), tyrosine hydroxylase (TH) and substance P (SP) have been studied in rat recto-anal region using quinacrine fluorescent, NADPH-d histochemical and ABC immunohistochemical methods respectively. Single ATP fluorescent nerve perikarya were observed in the myenteric ganglia of the rectum and anal canal. Numerous NADPH-d nerve cell bodies in myenteric ganglia of both investigated regions were found. Single nerve cell bodies immunoreactive for ChAT and SP were found in the myenteric ganglia of the rectum and anal canal. High number of immunoreactive nerve fibres in the myenteric ganglia and in the internodal strands were observed. Large number nerve fibres, demonstrating immunoreactivity for all three peptides in the muscle coat between the smooth muscle cells were observed. In the internal anal sphincter ChAT-, TH-, SP-immunoreactive and NADPH-d-positive varicose and folded nerve fibres were found.

Key words: adenosine triphosphate, nicotinamide adenine dinucleotide phosphate-diaphorase, choline acetyltransferase, tyrosine hydroxylase, substance P.

Introduction

The maintenance of continence is a complex process of interrelating factors which includes the sphincter muscles, rectal and anal sensation and stool composition. The regulation of activity of muscles in recto-anal region depends on the intrinsic myogenic properties and external innervation. Projections from nerves within the enteric nervous system serve to control the internal anal sphincter [2]. The localization and function of nerve types innervating the distal part of the intestinal canal has been intensively examined in view of their involvement in process of maintenance of the bowel continence [1, 4, 6, 10]. Generally, these studies investigate only the single neurotransmitter in norm [6] and in some pathological conditions, such as chronic constipation, anal fissure, Hirschprung's disease [5, 7].

The aim of this study was to investigate the presence, localization and distribution of the ATP, NADPH-d-, SP-, ChAT- and TH-positive nerve elements in the myenteric ganglia and in the muscle coat of rat recto-anal region.

Materials and Methods

All experimental procedures were carried out in agreement with the Bioethic Commission of the Institute of Neurobiology of the Bulgarian Academy of Sciences. To investigate ATP in myenteric ganglia, the quinacrine fluorescent technique of O l s s o n et al. [10] was used. The tissue sections were incubated in 10^{-6} quinacrine solution for 30 min at 37°C. Appropriate filter combination was applied. Under deep ether anesthesia 6 young male Wistar rats were perfused transcardially with 0.05 M phosphate buffered saline (PBS), pH 7.3, followed by a fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.3. The rectum with the anal canal and the internal anal sphincter were cut out and postfixed in the same fixative over night at 4°C. Then, the specimens were cryoprotected in 20% sucrose in PB. Thirty μm thick tissue sections were cut on "Reichert Jung" freezing microtome. The sections from muscle coat were collected in PBS and washed in the same solution over night. The histochemical staining procedure was performed according to the NADPH-diaphorase technique [11].

The immunohistochemical reaction was realized by using the avidin-biotin-peroxidase complex (ABC) technique of H s u et al. [3]. Mouse anti-TH monoclonal, rabbit anti-SP polyclonal and goat anti-ChAT polyclonal antibodies and respective (anti-mouse, anti-rabbit or anti-goat) IgG and ABC complex were used. Visualization of the reaction was made by 3,3'-DAB/H_2O_2 and nickel ammonium sulphate in some experiments was applied for magnification intensity of the reaction. Following the development of the reactions the sections were mounted on chrome-gelatin-coated slides, air dried, dehydrated in alcohol, cleared in xylene and embedded in Entellan. Control sections were processed by omission of the substrate β-NADPH and or respective primary antibody and the results were negative.

Results

Single quinacrine-fluorescent, numerous NADPH-d- (Fig. 1a), single SP- (Fig. 2a) and ChAT-positive (Fig. 3a) nerve cell bodies in the myenteric ganglia of the distal rectum and anal canal were observed. TH-immunoreactive neuronal perikarya in the myenteric ganglia of both regions were not found. Quinacrine-fluorescence nerve fibres were not presented. The immunoreactive nerve fibres were observed in the myenteric ganglia. The nerve fibres were of different sizes and had a characteristically varicose appearance. The number of SP-positive nerve fibres around the neurons was greater than the number of the other positive varicosities. The immunoreactive nerve fibers formed a network in the neuropil of the ganglia (Figs. 2a, 3a) and they could be traced for long distance in the internodal strands. NADPH-d- and peptide-positive fibres as single fibres or in nerve trunks run parallel to the muscle cells of the muscle layers. In the circular muscle layer they occurred more often than in the longitudinal one. In the internal anal sphincter NADPH-d- (Fig. 1b), SP- (Fig. 2b), ChAT- (Fig. 3b) and TH-immunoreactive nerve fibres were observed such as varicose, folded and run parallel to the smooth muscle cells. The NADPH-d-positive nerve fibres were more numerous than the peptide immunoreactive ones.

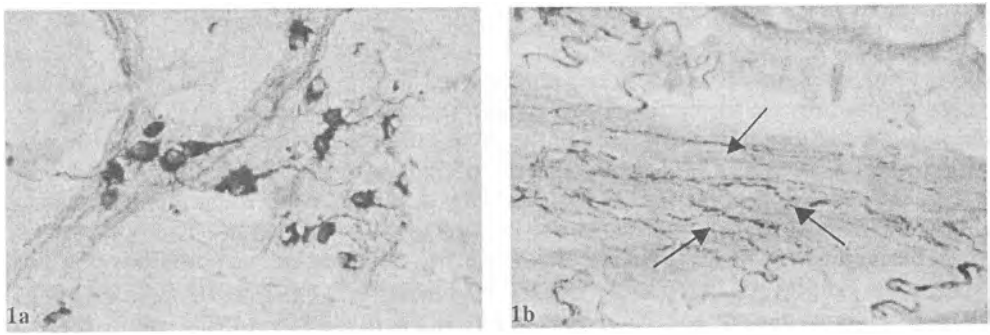

Fig. 1. NADPH-d-positive perikarya in myenteric ganglion of the anal canal (**a**) and positive, varicose and folded nerve fibres (arrows) in internal anal sphincter (**b**)

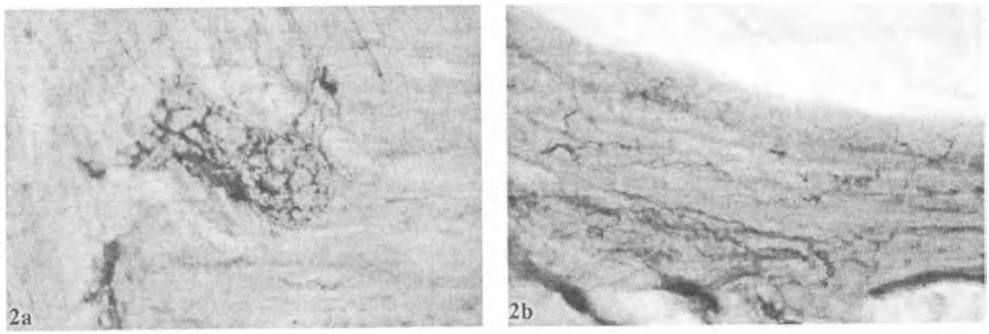

Fig. 2 **a** – SP-immunoreactivity in myenteric ganglion of the anal canal. Single SP-positive perikarya (head arrows) and varicose axons branching to form basket-like structures apparently around unlabeled myenteric nerve cell bodies (arrows); varicose and folded SP-positive nerve fibres (arrows) in internal anal sphincter (**b**)

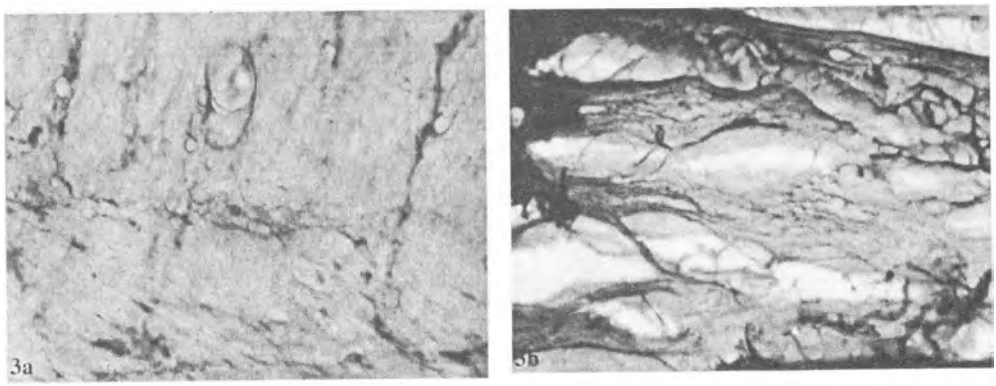

Fig. 3. ChAT-positive nerve perikarya (arrows) and varicose nerve fibres in myenteric ganglia of the distal rectum (**a**); positive nerve fibres (arrows) in internal anal sphincter (**b**); scale bar – 50 μm

Discussion

The myenteric plexus is a major target of nerves in the gut wall. The results of the present study provide further evidence concerning the existence of ATP-, NADPH-d- and peptide-positive nerve elements in the recto-anal region in rat model. Our results for distribution of NADPH-d-positive nerve perikarya in the recto-anal region are in agreement with the observations of O ` K e l l e y et al. [6]. We present the distribution of NADPH-d-positive nerve fibres in the internal anal sphincter also. The presence of single SP- and ChAT-positive perikarya in the myenteric ganglia and numerous positive axons in the ganglia and in muscle layers did not exclude of extrinsic origin. In our study the lack of TH-immunoreactive nerve perikarya in the myenteric ganglia but numerous positive axons suggests that these axons could be of extrinsic origin, probably of postglionic sympathetic neurons. This finding corresponds to the data of O l s s o n et al. [9], received by using combined tract tracing and immunohistochemical studies.

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