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Vesicular Amine Transporter VMAT2 in the Gut: from Principal Mechanism to Therapeutic Application

I. Stoyanova

Department of Anatomy, Faculty of Medicine, Thracian University, Stara Zagora

Besides the role of classical neurotransmitter, histamine plays a key role in the immune/inflammatory processes in the gastrointestinal tract. Specific transport proteins pack the amine neurotransmitters into vesicles so that their release can be regulated by neural activity. Recently, two vesicular amine transporters (VMAT1 and VMAT2), essential components of monoaminergic neurons and endocrine cells were identified. In our study we investigated VMAT2 distribution in rat small intestine using immunocytochemical techniques. VMAT2-immunreactivity was found in neurons of the submucosal plexus. Nerve fibres containing VMAT2 were numerous in the submucosal and myenteric plexuses, in the circular muscle layer and around the blood vessels. Some fibres were observed beneath the epithelial cells.

This data provide important information about the amine-handling structures in the gut wall, and neuroendocrine and immune/inflammatory cell functions. Moreover, it raises the possibility for development of new pharmacotherapeutic approach to inflammatory bowel diseases.

Key words: gut innervation, histamine transporters, immunohistochemistry, rat.

Introduction

Besides the function of classical neurotransmitter in the nervous system, the biogenic amine histamine (HIS) also plays a key role in the endocrine and immune/inflammatory system. In the gastrointestinal tract (GIT) HIS is a crucial mediator, and during the last two decades it was discovered to be responsible also for diarrhoea in inflammatory bowel diseases (IBD) and food allergy [1].

HIS-handling cells, like the other amine-handling cells, are characterized by synthesis, accumulation and secretion of the amine, which require amine-synthesizing enzymes, plasma membrane transporters for amine intake, and intracellular transporters, named vesicular amine transporters (VATs), for amine loading from the cytoplasm into secretory or synaptic vesicles [2]. Two structurally related but pharmacologically distinct monoamine transporters are known: VMAT1 and VMAT2 [3]. They act as an electogenic exchanger of protons and monoamines, using a proton electrochemical gradient. Functional analysis showed that the two VATs differ in substrate recognition and inhibition by drugs. [4]. In addition, VMAT1 and VMAT2 differ in their tissue distribution. While in the rat VMAT1 is a principal amine transporter of the PNS and of the neuroendocrine and mast cells, VMAT2 is expressed

predominantly in the neuronal amine-handling cells: serotoninergic, noradrenergic, dopaminergic, histaminergic and adrenergic neurons of the CNS [5].

In both rodent and human, most of the VMAT2 positive nerve fibres are found at the blood vessels' wall and around the enteric ganglia. These fibres represent projections of postganglionic sympathetic neurons, and in the muscle and mucosal layer they are rare. VMAT2 positive neuronal perikarya are very rare or absent in the rodent gut [5].

Despite the fact that the biogenic amine-containing neuronal structures in the enteric nervous system (ENS) were extensively studied, there are still several unanswered questions about their distributional pattern, chemical coding and function. Therefore, we aimed this study to demonstrate the VMAT2-positive elements in the intestinal wall of the rat, and to assess their potential therapeutic implication.

Material and Methods

Specimens from the distal ileum of five rats were investigated with immunocytochemical techniques for detection of VMAT2. The staining was performed using the avidin-biotin method on free-floating sections with primary antibody rabbit anti-VMAT2, for 24 h at room temperature. After rinsing in PBS, sections were incubated with the secondary antibody, biotinylated goat anti-rabbit IgG. After washing the sections, the ABC complex was applied. Following rinsing, peroxidase activity was visualized with the SG chromogen as gray staining of the immunoreactive structures. Finally, sections were dehydrated in a graded series of alcohol and xylene, and embedded in Entellan.

Results

Immunostaining of VMAT2 in specimens of rat distal ileum showed abundant varicose neuronal fibres in all layers of the gut wall. As shown in Fig. 1, VMAT2-immunreactivity was found in single neurons of the submucosal and myenteric plexuses. The immunoreactive fibres clearly marked by the SG were numerous in both plexuses, and they surrounded VMAT2-positive neuronalal perikarya. In addition, we observed a high frequency of VMAT2-positive varicosities in the circular muscle layer and around the blood vessels. In the mucosa there was well expressed VMAT2-immunoreactivity. Some immunolabelled nerve fibres were observed distributed throughout lamina propria. As can be seen in Fig. 2, a large number of VMAT2-containing neuronal processes and terminals were in a close apposition to the intestinal glands, and delineated the glandular epithelial cells.

Discussion

In the present study, we show that the intestinal wall of the rat is well innervated by VMAT2 expressing intrinsing neurons and neuronal fibres. Moreover, it clearly demonstrates abundance of VMAT2-positive neuronal projections into the intestinal musculature and mucosa layers; hence, their function is under monoamine-ergic control.

These data provide important information about the amine-handling neuronal structures in the gut. VATs-staining will enable co-transmitters to be identified, particularly in subtypes of intestinal amine-handling cells. The visualization of the

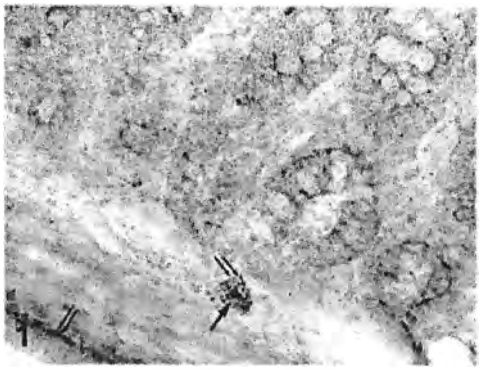

Fig. 1. Photomicrograph of the distal ileum showing VMAT2-positive neuron in the submucosal plexus (arrow). Note the numerous VMAT2-immunoreactive neuronal fibres in both the myenteric and submucosal plexus (double arrows). × 400

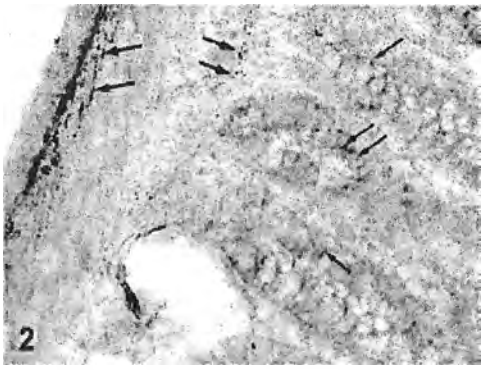

Fig. 2. Numerous neuronal projections, stained for VMAT2 in the circular muscle layer and in the submucosis (thick arrows). The glandular epithelium is delineated by VMAT2-containing varicosities (thin arrows). × 400

VMAT2-positive elements of the gut wall provides a potential for their imaging in the clinical context of disorders, related to them. This immunocytochemical method allows an *in situ* exploration of plasticity, regulation, and degeneration of specific sets of amine-handling neurons, and the function of amine-handling inflammatory and immune cells.

The abundance of VMAT2-positive nerve fibres in the intestinal wall and the fact that HIS transporter VMAT2 can be inhibited by substances like ketanserin, reserpin or dicyclohexylcarbodiimide (DCCD), which clock various H^+-translocating enzymes [6], suggest that HIS or VMAT2 antagonists may be used for pharmacological targeting of IBD. Moreover, it raises the possibility for development of new pharmacotherapeutic approach to inflammatory bowel diseases.

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