Research

Vesicular Amine Transporter VMAT2 in the Rat Ileum: from Principal Mechanism to Clinical Applications

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Abstract

Besides the role of a 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 the rat distal ileum using an immunocytochemical technique. VMAT2-immunoreactivity was found in neurons of the submucosal and myenteric plexuses, as well as in a dense network of nerve fibers. VMAT2-containing varicosities were numerous in the circular muscle layer and around the blood vessels. Some fibers and nerve terminals were observed beneath the epithelial cells. This data provide important information about the amine-handling structures in the distal gut. Moreover, it raises the possibility for development of new pharmacotherapeutic approach to inflammatory bowel diseases.

Keywords: Gut innervation, Histamine transporters, VMAT2, Immunohistochemistry, Rat

Introduction

Besides the function of a 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 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 electrogenic 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 [4,5]. VMAT2 has two to threefold higher affinity for most monoamine substrates than VMAT1 [6]. 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 [7,8].

In humans, Erickson and collaborators [5] have demonstrated that all the chromogranin A-positive enterochromaffin (EC) cells of the stomach are VMAT2-positive, while in contrast, the majority of the intestinal EC cells are VMAT1-positive but VMAT2-negative. VMAT2 staining has been observed in the principal ganglion cells of the sympathetic nervous system, including the adrenal medulla [5]. In both rodent and human gut, most of the VMAT2 positive nerve fibers are found at the blood vessels' wall and around the enteric ganglia [8]. These fibers represent projections of postganglionic sympathetic neurons. They are rare in the muscle and mucosal layers. According to Weihe and Eiden [7], VMAT2 positive neuronal perikarya are very rare or absent in the rodent gut, while Burger et al. have demonstrated them in the submucosal and myenteric plexus [9].

Unlike the GABA and glutamate vesicular transporters, which are predominantly axonal [10], VMAT2 is expressed in the cell bodies and dendrites, as well as in axon terminals of monoamine neurons [11]. In axons, VMAT2 resides in synaptic vesicles and large dense-core vesicles (LDCVs) [12]. In dendrites and neuronal perikarya, the transporter is localized on tubulovesicular structures, and it is still unclear whether these membranes undergo regulated exocytosis [13].

Despite the fact that the biogenic amine-containing neuronal elements in the enteric nervous system (ENS) were quite extensively investigated, there are still some contradictions and several unanswered questions about their distributional pattern, chemical coding and function. Therefore, with this study we aimed at demonstrating VMAT2-ergic innervation of the rat intestine. Additionally, we discuss their potential role in the etiopathogenesis of some diseases and prospective therapeutic implications.

Material and Methods

Animals and tissue preparation

Seven adult Wistar rats (250-359 g body weight) were used in this study. All housing facilities and procedures used were approved by the Animal Care and Use Committee of the University of Twente, and were carried out in accordance with the European Communities Council Directive of 24 November 1986. The rats were anesthetized and perfused with 4% paraformaldehyde in 0.1 M PBS (pH 7.4). Specimens from the distal ileum were post fixed by immersion in the same fixative for 24 h at 4°C, and cryoprotected overnight in 25% sucrose in PBS at 4°C. They were embedded in TissueTek OCT compound (Miles Inc., Elkhart, IN, USA), frozen, and 30 µm thick sections were cut in a cryostat at -20°C. The specimens were investigated with immunocytochemical techniques for detection of VMAT2.

Immunocytochemistry

The immunostaining was performed according to the ABC (abiding-biotin-horseradish peroxidase) method [14]. Briefly, the endogenous peroxidase was
inactivated with hydrogen peroxide (0.3% in methanol/PBS for 30 min), and the background was blocked with 5% normal goat serum (NGS) and 1% bovine serum albumin (BSA) in 0.5% Triton X-100. Appropriate rinsing in PBS followed these and the subsequent procedures. Incubation in the primary antibodies (rabbit anti-VMAT2 IgG, Sigma-Aldrich, St. Louis, MO, USA) was for 20 h at room temperature (RT), followed by treatment for 2 h with biotinilated secondary antibody (goat anti-rabbit IgG, 1:500; Jackson ImmunoResearch, West Grove, PA, USA). After rinsing, the slices were incubated for 1 h in ABC complex (Vector Labs, Burlingame, CA, USA, 6.25 µl/ml of each compound in PBS). The peroxidase activity was visualized using 2.4% SG substrate kit (Vector) in PBS for 5 minutes, at room temperature. All specimens were counterstained with 0.5% Neutral red (Sigma-Aldrich). Finally, the slices were dehydrated in alcohol, cleared in xylene, and coverslipped with Entellan (Merck, Darmstadt, Germany).

Negative controls included incubation at 4°C for 24 h after antigen-antibody preabsorption with the native antigen SLC18A2 (Sigma-Aldrich), and replacement of the primary antibody with normal serum at the same concentration.

**Results**

In the distal ileum of rat the immunostaining for VMAT2 detection showed a small subpopulation of VMAT2-positive intrinsic enteric neurons in both the submucosal and myenteric plexuses (Figures 1-3). The staining revealed clearly distinguishable granular cytoplasmic pattern in those neurons. Additionally, they were surrounded by VMAT2-immunolabelled nerve fibers (Figure 2). Varicose nerve fibers, which contain a high density of synaptic vesicles bearing the VMAT2 were present in all layers of the gut wall, markedly numerous in both the submucosal and myenteric plexuses (Figures 3 and 4). A high number of VMAT2-immunoreactive (IR) projections were found in the circular muscle layer (Figure 4). VMAT2-IR fibers formed a dense perivascular networks, particularly in the submucosa (Figure 5). In the mucosa, VMAT2-IR nerve fibers were distributed throughout the whole lamina propria, and outlined the intestinal glands (Figure 6).

A large number of those fibres and terminals were in a close apposition to the glandular epithelial cells and to the smooth muscle cells of the intestinal villi (data not shown). No VMAT2-IR was found in the mucosal enteroendocrine (EC) cells of the ileum.

**Figure 1:** Transverse section of the gut showing VMAT2-immunostaining in numerous varicose neuronal fibers in both the myenteric and submucosal plexus, and in all layers of the intestinal wall. X 400

**Figure 2:** Photomicrograph of the distal ileum showing a VMAT2-positive neurone in the submucosal plexus (arrow). Note the numerous VMAT2-immunoreactive neuronal fibers in both the myenteric and submucosal plexuses (double arrows). X 400
Discussion

The results from this study show that the rat distal ileum is innervated by VMAT2-expressing neuronal components. Our finding of small number of intrinsic monoaminergic neurons is in consent with the previously reported 1-2% of VMAT2-positive ganglionic cells in the lower part of the rat intestine [15,16]. These neurons have been identified as serotonergic phenotype [17]. In the low part of the human gut, the mucosal and smooth muscle layers show very sparse VMAT2-labeled nerve fibers [15], however in the rat, as previously demonstrated [18] and also confirmed by this study, VMAT2-IR varicosities are quite abundant. It suggests that in rodents, the propulsive motor activity, secretion and active ion transport are under catecholaminergic control. Moreover, unlike the porcine ileum, where VMAT2-IR neuronal processes do not innervate blood vessels [19], in the rat we found quite abundant VAMT2-IR vascular innervations. This could be due to interspecies differences or differences in the staining procedures. The perivascular VMAT2-positive networks indicate that the mucosal blood flow is regulated by monoamines as well. Apparently, part of those neuronal fibres
originates from the intrinsic amine-handling ganglionic cell, however the other, particularly those innervating the blood vessels, probably represent terminals of postganglionic sympathetic (noradrenergic) neurons [8,18,20].

Additionally, amine-handling components of the ENS may also participate in some aspects of inflammation and immunity during pathological conditions [21]. The abundance of VMAT2-positive nerve fibers in the intestinal wall and the fact that HIS transporter VMAT2 can be inhibited by substances like ketanserin, reserpine or dicyclohexylcarbodiimide (DCCD), which clock various H⁺-translocating enzymes [22], suggest that HIS or VMAT2 antagonists may be used for pharmacological targeting of inflammatory bowel diseases. Moreover, it raises the possibility for development of new pharmacotherapeutic approach to them.

The visualization of VMAT2-positive elements of the gut wall provides a potential for their imaging in the clinical context of disorders, related to them, particularly intestinal tumours. Since western blotting is not able to detect VMAT2 in normal ileum and colon, most likely due to the small number of VMAT2-expressing structures in them, the immunocytochemistry thus appears to be the most sensitive method with diagnostic potential in tumour pathology [23]. This method also allows an in situ exploration of plasticity, regulation, and degeneration of specific sets of amine-handling neurones, and the function of amine-handling inflammatory and immune cells.

**Conflict of Interest:** None to disclose

**References**


