Role of Serotonin 5-HT<sub>3</sub> Receptors in Intestinal Inflammation

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Serotonin (5-hydroxytryptamine; 5-HT<sub>3</sub>), a well-characterized neurotransmitter in the central nervous system, plays a crucial role in regulating mood, body temperature, sleep, appetite, and metabolism. Serotonin is synthesized in the serotonergic neuron of the central nervous system; however, approximately 90% of serotonin is synthesized and localized in the gastrointestinal (GI) tract, especially in the enterochromaffin (EC) cells. In the GI tract, serotonin mediates control over a variety of physiological functions such as contraction/relaxation of smooth muscle, and peristaltic and secretory reflexes, directly or indirectly through intrinsic primary afferent neurons. The receptors mediating the action of serotonin are mainly classified into 7 major groups known as the 5-HT<sub>1</sub> to 5-HT<sub>7</sub> receptors. The 5-HT<sub>3</sub> receptor is distinguished from among the other 5-HT receptor subtypes because it is only a ligand-gated ion channel, whereas the other subtypes serve as G protein-coupled receptors. The 5-HT<sub>3</sub> receptor, which is generally considered to be localized in the brain, and peripheral nervous systems and mediates a variety of physiological functions such as contraction/relaxation of smooth muscle, and peristaltic and secretory reflexes, directly or indirectly through intrinsic primary afferent neurons. The receptors mediating the action of serotonin are mainly classified into 7 major groups known as the 5-HT<sub>1</sub> to 5-HT<sub>7</sub> receptors. The 5-HT<sub>3</sub> receptor is distinguished from among the other 5-HT receptor subtypes because it is only a ligand-gated ion channel, whereas the other subtypes serve as G protein-coupled receptors. The 5-HT<sub>3</sub> receptor, which is generally considered to be localized in the brain, and peripheral nervous systems, is involved in processes associated with emotion, cognition, memory, pain perception, and GI functions including secretion and motility. Recently, an increasing number of findings have provided evidence of the important role of the 5-HT<sub>3</sub> receptor in the regulation of inflammatory and immune responses. In fact, several 5-HT<sub>3</sub> receptor antagonists have been reported to ameliorate intestinal inflammation. Therefore, this review focuses on the role of 5-HT<sub>3</sub> receptors in the pathogenesis of intestinal inflammation.

Key words serotonin; 5-HT<sub>3</sub> receptor; intestinal; inflammation

1. EXPRESSION AND FUNCTION OF 5-HT<sub>3</sub> RECEPTORS IN THE GASTROINTESTINAL TRACT

The 5-HT<sub>3</sub> receptor subtype is a member of the Cys-loop ligand-gated cation channels (Na<sup>+</sup>, Ca<sup>2+</sup>, and K<sup>+</sup>), such as the nicotinic acetylcholine (nACh), and the Zn<sup>2+</sup>-activated ion channel (ZAC).<sup>1</sup> This receptor is a pentamer of subunits that assemble pseudosymmetrically to form a central pore of an ion channel.<sup>1,2</sup> To date, five 5-HT<sub>3</sub> receptor subunits have been cloned, namely, 5-HT<sub>3A</sub>, 5-HT<sub>3B</sub>, 5-HT<sub>3C</sub>, 5-HT<sub>3D</sub>, and 5-HT<sub>3E</sub>. Only one subunit, 5-HT<sub>3A</sub>, can form functional homomeric receptors (homopentamer), whereas the other 4 subunits form functional heteromeric receptors together with 5-HT<sub>3A</sub> subunits (heteropentamer).<sup>1,3</sup> Although the ubiquitous expression of all the subunits have been observed in dorsal root ganglia, the brain, and gastrointestinal (GI) tract,<sup>4</sup> the specific localization of each 5-HT<sub>3</sub> subunit is still controversial.

The 5-HT<sub>3</sub> receptor is expressed throughout the central and peripheral nervous systems and mediates a variety of physiological functions. In the intestine, 5-HT<sub>3</sub> receptors are expressed in the mucosal cell layer and neuronal cell bodies of the submucosal and myenteric plexuses.<sup>5</sup> Based on these expression patterns, the 5-HT<sub>3</sub> receptor is considered to play an important role in the regulation of autonomous functions including motility and peristalsis, secretion, and visceral per-ceptation,<sup>6</sup> and may contribute to functional GI disorders such as dyspepsia, gastroesophageal reflux disease (GERD), and irritable bowel syndrome (IBS). Several studies have recently demonstrated that 5-HT<sub>3</sub> receptors are expressed extraneuronally in immune and inflammatory cells such as monocytes,<sup>7</sup> T cells,<sup>8,9</sup> and dendritic cells,<sup>10</sup> as well as macrophage-like synovial cells.<sup>10</sup> These new findings suggest that serotonin/5-HT<sub>3</sub> receptor pathways may be associated with immune and inflammatory responses, and may be involved in inflammatory intestinal diseases.

2. PATHOGENIC ROLE OF ENDOGENOUS SEROTONIN IN INTESTINAL INFLAMMATION

Several studies have demonstrated an increase in the number of serotonin-producing EC cells and serotonin level in the colon of patients with inflammatory bowel disease<sup>12</sup> and in experimental models of colonic inflammation.<sup>13–15</sup> Thus, endogenous serotonin may be implicated in the pathogenesis of intestinal inflammation. In fact, Ghia et al.<sup>19</sup> recently reported that the severity of colitis that was experimentally induced by dextran sulfate sodium (DSS) and trinitrobenzen sulfonic acid (TNBS) was significantly decreased in mice deficient in tryptophan hydroxylase-1 (TPH-1), an enzyme catalyzing serotonin synthesis. They further showed that the depletion of endogenous serotonin induced by pretreatment with p-chlorophenylalanine (PCPA), an inhibitor of TPH,
Similarly attenuated the severity of colitis. These findings suggest the pathogenic role of endogenous serotonin in intestinal inflammation. Similarly, we recently reported that a small intestinal injury induced by indomethacin, a non-steroidal anti-inflammatory drug (NSAID), accompanied by an increase in myeloperoxidase (MPO) activity, inducible nitric oxide synthase (iNOS) level, and inflammatory cytokine expression, was strongly suppressed by pretreatment with PCPA.\(^7\) We further demonstrated that plasma serotonin level was significantly increased after the administration of indomethacin, and that this response was potently prevented by pretreatment with PCPA. Several studies revealed that the release of serotonin from enterochromaffin (EC) cells was increased by inflammatory responses, including inflammatory cytokines,\(^{14,18}\) and bacterial infection.\(^{19}\) Therefore, endogenous serotonin released from EC cells in response to an inflammatory response may play a pathogenic role in intestinal inflammation.

3. ANTI-INTESTINAL INFLAMMATORY EFFECTS OF THE 5-HT\(_3\) RECEPTOR ANTAGONISTS

Several 5-HT\(_3\) receptor antagonists have recently been demonstrated to be useful for various intestinal inflammatory diseases (Table 1). Tropisetron and granisetron exerted beneficial effects in acetic acid-induced colitis in rats.\(^{20,21}\) These antagonists inhibited the increased production of inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF-\(\alpha\)) in response to an intracolorectal injection of acetic acid; however, the role of 5-HT\(_3\) receptors in these anti-inflammatory actions is unclear. More recently, ondansetron was demonstrated to have an anti-inflammatory effect on TNBS-induced colitis in rats.\(^{22}\) It is interesting that the preventive effect of ondansetron on TNBS-induced colitis was neutralized by \emph{meta}-chlorophenyl-biguanide (mCPBG), a specific agonist of 5-HT\(_3\) receptors. This finding strongly suggests the pathogenic role of 5-HT\(_3\) receptors in TNBS-induced colitis. Similarly, we investigated the effect of various subtype-specific antagonists of 5-HT receptors on small intestinal lesions induced by indomethacin to determine the association of 5-HT receptor subtypes with endogenous serotonin-related intestinal inflammation.\(^7\) Several factors such as enterobacteria,\(^{23,24}\) neutrophils,\(^{25}\) nitric oxide (NO) derived from iNOS,\(^{23,24}\) and inflammatory cytokines,\(^{25}\) as well as prostaglandin deficiency, are reportedly involved in the pathogenesis of NSAID-induced small intestinal lesions. NAN-190, ketanserin, and SB269970, at doses sufficient to block the responses \(\alpha\) the 5-HT\(_1\), 5-HT\(_2\), and 5-HT\(_7\) receptors, respectively, did not affect the occurrence of these lesions. In contrast, ondansetron, an antagonist of 5-HT\(_3\) receptors, dose-dependently suppressed the severity of these intestinal lesions. A similar suppressive effect was observed with ramuciren, a more potent and selective 5-HT\(_3\) receptor antagonist.\(^{26}\) These findings strongly suggest that the 5-HT\(_3\) receptors play a critical role in the development of indomethacin-induced small intestinal lesions. We observed that GR113808, a 5-HT\(_3\) receptor antagonist, significantly aggravated the severity of these lesions. Thus, it is interesting that endogenous serotonin has an intriguing dual role in the pathogenesis of indomethacin-induced small intestinal lesions, dependent on 5-HT receptor subtypes. Furthermore, we recently reported that ramuciren and ondansetron significantly suppressed the severity of intestinal mucositis induced by 5-fluorouracil (5-FU), an anticancer agent.\(^{27}\) The pathogenesis of 5-FU-induced intestinal mucositis remains unclear, but is considered to be a consequence of various processes, including abnormal inflammation and apoptosis, together with cellular hypoproliferation and direct cytotoxicity.\(^{28}\) We demonstrated that apoptosis in the intestinal crypt was caused by the upregulation of TNF-\(\alpha\)-induced caspase-3/8 activations.\(^{29}\) Ramuciren and ondansetron clearly suppressed the upregulation of TNF-\(\alpha\) expression and subsequent caspase-3/8 activations in the intestinal crypt in response to 5-FU. These numerous findings provide evidence

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Other anti-inflammatory effects

| Tropisetron                    | CLP-induced sepsis       | TNF-\(\alpha\), IL-6, sympathetic over-stimulation ↓ | Setoguchi \textit{et al.}\(^{26}\) |
| Granisetron                    | Carrageenan-induced air pouch | TNF-\(\alpha\), PGE2 ↓ | Maleki-Dizaji \textit{et al.}\(^{12}\) |
| Tropisetron                    | LPS-stimulated TNF-\(\alpha\) and IL-1 production in monocytes | — | Fiebich \textit{et al.}\(^{7}\) |
| Ondansetron                    | LPS-activated TNF-\(\alpha\) and IL-1 production in monocytes | p38 MAP kinase ↓ | Stratz \textit{et al.}\(^{30}\) |
| Tropisetron                    | SEB- and PMA-stimulated IL-2 expression and T cell functions in T cells | Calcineurin pathway ↓ | Vega \textit{et al.}\(^{31}\) |
| Ondansetron                    | Serotonin-induced overexpression ofPGE2 in macrophage-like synovial cells | — | Seidal \textit{et al.}\(^{11}\) |
that suggests that various 5-HT₃ receptor antagonists suppress intestinal inflammatory diseases.

4. MECHANISMS OF THE ANTI-INFLAMMATORY EFFECTS OF THE 5-HT₃ RECEPTOR ANTAGONISTS

The mechanism by which the 5-HT₃ receptor antagonists produce intestinal anti-inflammatory effects remains unclear. As mentioned previously, an increasing number of findings provide evidence that suggests the role of 5-HT₃ receptors expressed in immune and inflammatory cells including monocytes, T cells, and dendritic cells, as well as macrophage-like synovial cells, in the regulation of inflammatory processes. Lipopolysaccharide-stimulated production of TNF-α and IL-1 was inhibited by tropisetron and ondansetron in human monocytes. Tropisetron and ondansetron can inhibit IL-2 expression and T-cell activation via suppression of the calcineurin pathway. However, granisetron, an equipotent antagonist of 5-HT₃ receptors, along with ondansetron, failed to inhibit T-cell functions, suggesting that this action of tropisetron and ondansetron is independent of 5-HT₃ receptors. Furthermore, the anti-inflammatory effect has been shown to be mediated by the inhibition of p38 mitogen-activated protein kinase expression, independent of the 5-HT₃ receptors. In contrast, serotonin stimulated prostaglandin E₂ production in macrophage-like synovial cells via activation of the 5-HT₂A and 5-HT₃ receptors. Granisetron has been shown to attenuate the inflammatory responses, including inflammatory cytokine production, in carrageenan-induced air pouch models. We also recently reported that both ramosetron and ondansetron significantly suppressed the severity of intestinal injury induced by indomethacin and 5-FU, ramosetron being 10 times more potent than ondansetron. Ramosetron is a specific and potent antagonist of 5-HT₃ receptors and was found to be 3–10 times more potent in inhibiting intestinal propulsion activity than ondansetron in vivo experiments. Thus, the suppressive effect of these 2 antagonists on intestinal injury may be dependent on the antagonistic activity of the 5-HT₃ receptors. We performed further immunohistochemical studies that demonstrated the expression of the 5-HT₃ receptors in certain cells localized in the lamina propria of intestinal villi. Of interest is that some cells expressing 5-HT₃ receptors were positive for CD11b, a marker of macrophage-like cells, and for TNF-α. These findings suggest that 5-HT₃ receptors are expressed in inflammatory cells, such as macrophages localized in the small intestine, and enhance the inflammatory responses in association with indomethacin and 5-FU-induced small intestinal injuries.

Expression of the 5-HT₃ receptors was reportedly detected not only in cell-like structures but also in nerve fibers of colonic mucosa. It is interesting that the expression levels of the 5-HT₃ receptors in nerve fibers of colonic mucosa were significantly increased in DSS-induced colitis. Therefore, the anti-inflammatory effects of the 5-HT₃ receptor antagonists on intestinal inflammation is assumed to be partly mediated by neuronal pathways. However, further studies are needed to clarify this hypothesis.

5. CONCLUSION

The 5-HT₃ receptor antagonist was first developed in the 1980s and has since been used for the prevention and treatment of chemotherapy-induced emesis. Recent findings on the role of 5-HT₃ receptors in immune and inflammatory responses have provided evidence of their beneficial effects on various inflammatory diseases. In particular, trospisetron was demonstrated to be consistently effective as a corticosteroid therapy for rheumatoid diseases in humans. Therefore, serotonin/5-HT₃ receptor pathways offer new knowledge on the regulation of immune and inflammatory responses, and the 5-HT₃ receptor antagonists may be a new class of anti-inflammatory drugs. In addition, 5-HT₃ receptor antagonists may be considered a relatively safe treatment for inflammatory diseases of the GI tract, compared with NSAIDs and corticosteroids. Thus, further investigations are needed to determine the role of serotonin/5-HT₃ receptors in inflammatory and immune responses in the GI tract.

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