Pharmacological Research 66 (2012) 226–234

Contents lists available at SciVerse ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Dual role of serotonin in the pathogenesis of indomethacin-induced small intestinal ulceration: Pro-ulcerogenic action via 5-HT3 receptors and anti-ulcerogenic action via 5-HT4 receptors

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ARTICLE INFO

Article history:
Received 6 April 2012
Received in revised form 4 June 2012
Accepted 4 June 2012

Keywords:
Dual role of serotonin
5-HT receptor subtyeps
Indomethacin-induced intestinal lesions
Ondansetron
Mosapride

ABSTRACT

Serotonin (5-HT) exerts multiple physiological functions not only in the central and peripheral nervous systems but also in the gastrointestinal tract, and these multiple functions are accounted for by a variety of 5-HT receptor subtypes. We investigated the role of 5-HT in the pathogenesis of indomethacin-induced intestinal lesions in mice, in relation to 5-HT receptor subtypes. A single oral administration of indomethacin (10 mg/kg) provoked damage in the small intestine of mice 24 h later, and this response was prevented by pretreatment with *p*-chlorophenylalanine (a 5-HT synthesis inhibitor). The administration of 5-HT3 receptor antagonists, such as ondansetron and ramosetron, dose-dependently reduced the severity of the intestinal lesions, whereas a high dose of GR113808 (a 5-HT4 receptor antagonist) significantly aggravated these lesions. In contrast, NAN-190 (a 5-HT1 receptor antagonist), ketanserin (a 5-HT2 receptor antagonist), and SB269970 (a 5-HT7 receptor antagonist) had no effect on these lesions. Mosapride (a 5-HT4 receptor agonist) significantly reduced the severity of indomethacin-induced intestinal lesions, and this protective effect was totally prevented by either GR113808 or methyllycaconitine (an α7-nicotinic acetylcholine receptor antagonist). Indomethacin increased the activity of myeloperoxidase and the expression of inducible nitric oxide synthase, inflammatory cytokines, and chemokines in the small intestine; these responses were significantly attenuated by ondansetron and mosapride. These findings suggest that endogenous 5-HT exerts a dual role in the pathogenesis of indomethacin-induced intestinal lesions: pro-ulcerogenic action via 5-HT3 receptors and anti-ulcerogenic action via 5-HT4 receptors, and the latter effect via 5-HT4 receptors may be mediated by activation of α7-nicotinic acetylcholine receptors.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), commonly prescribed for the treatment of various inflammatory disorders, including rheumatoid arthritis and osteoarthritis, induce damage to both the stomach and the small intestine [1–3]. Recent clinical studies using capsule and double-balloon endoscopes have confirmed that NSAID-induced small intestinal lesions occur much more frequently than that previously estimated [4,5]. Indeed, approximately 10% of healthy subjects exhibit NSAID-induced small intestinal lesions, and the incidence became much greater, about 70%, in chronic NSAID users [5,6].

Several factors, such as enterobacteria [2,3,7], neutrophils [3,8], nitric oxide (NO) derived from inducible NO synthase (iNOS) [2,3,9], inflammatory cytokines, and chemokines [10–12], as well as prostaglandin deficiency [13,14], are reportedly involved in the pathogenesis of NSAID-induced small intestinal lesions. However, the pathogenic mechanisms of these lesions remain to be fully understood, and no satisfactory means for the prevention and treatment of these lesions are currently available, except for the use of prostaglandin analogs [15].

Serotonin (5-hydroxytryptamine; 5-HT), a well-characterized neurotransmitter in the central nervous system, plays a crucial

Abbreviations: 5-HT, serotonin; NSAID, non-steroidal anti-inflammatory drug; NO, nitric oxide; iNOS, inducible nitric oxide synthase; EC, enterochromaffin; GI, gastrointestinal; TPH-1, tryptophan hydroxylase-1; PCPA, *p*-chlorophenylalanine; PCR, polymerase chain reaction; TNF-α, tumor necrosis factor-α; KC, keratinocyte-derived chemokine; MIP-2, macrophage inflammatory protein-2; MCP-1, macrophage chemotactic protein-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL, interleukin.

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http://dx.doi.org/10.1016/j.phrs.2012.06.002

role in regulating mood, body temperature, sleep, appetite, and metabolism [16]. Although some of 5-HT is synthesized by the serotonergic neurons in the central nervous system, approximately 90% of 5-HT is synthesized and localized in the gastrointestinal (GI) tract [16,17], especially in the enterochromaffin (EC) cells [17,18]. 5-HT mediates control over a variety of physiological functions in the GI tract, such as contraction/relaxation of smooth muscle, and peristaltic and secretory reflexes, directly or through intrinsic primary afferent neurons [16,19,20]. Receptors mediating the action of 5-HT are now classified into 7 major groups, termed 5-HT1 to 5-HT7 receptors, which further include several subgroups [21].

Several studies demonstrated an increase in intestinal EC cell numbers and 5-HT content in patients with inflammatory bowel disease (IBD) [22] and in experimental models of colonic inflammation [23–26]. Ghia et al. [27] further showed that the severity of experimentally induced colitis was significantly decreased in mice deficient in tryptophan hydroxylase-1 (TPH-1), an enzyme catalyzing 5-HT synthesis, and those pretreated with *p*-chlorophenylalanine (PCPA), an inhibitor of TPH, suggesting a role of 5-HT in the pathogenesis of colitis. However, the role of 5-HT in the pathogenesis of NSAID-induced small intestinal lesions remains unexplored.

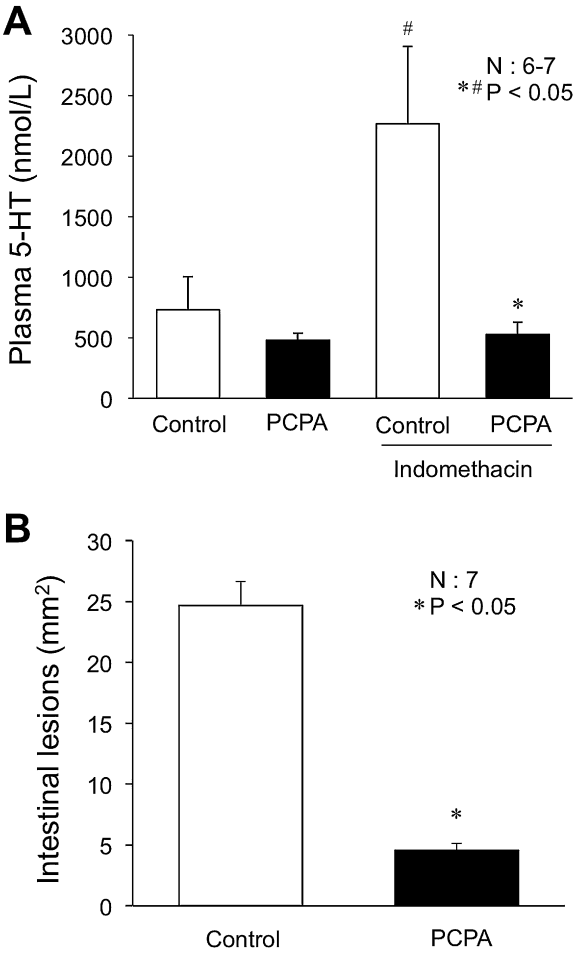

Fig. 1. Effects of PCPA on plasma 5-HT levels and indomethacin-induced intestinal lesions in mice. Animals were given indomethacin (10 mg/kg, p.o.) and sacrificed 24 h later. PCPA (300 mg/kg) was given i.p. 4 times, 48 h, 24 h, and 0.5 h before and 8 h after the administration of indomethacin. Blood samples were collected 8 h after the administration of indomethacin from the inferior vena cava, and the concentration of 5-HT was determined using an enzyme immunoassay. Data are presented as the mean ± S.E.M. for 6–7 mice. Significant differences at $P < 0.05$; *from control (vehicle alone); #from normal (indomethacin-untreated). (A) Plasma 5-HT levels and (B) intestinal lesions.

The aim of the present study was to investigate the role of endogenous 5-HT in the pathogenesis of indomethacin-induced small intestinal lesions using PCPA and various 5-HT receptor antagonists and agonists. We also examined the effect of 5-HT receptor antagonists and agonists on various pathogenic elements of these lesions, including activation of neutrophils, and upregulation of iNOS, inflammatory cytokines, and chemokines expressions.

2. Methods

2.1. Animals

Male C57BL/6J mice (20–23 g, SLC Co., Shizuoka, Japan) were acclimated to standard laboratory conditions (12-h light–dark cycle, temperature 22 ± 1 °C). The experiments were carried out using 5–9 mice per group. All experimental procedures were approved by the Experimental Animal Research Committee of Kyoto Pharmaceutical University.

2.2. Reagents

Drugs used were indomethacin, PCPA, NAN-190, GR113080, methyllycaconitine (Sigma–Aldrich, St. Louis, MO, USA), ketanserin (LKT Laboratories, St. Paul, MN, USA), ondansetron, SB269970 (Tocris Bioscience, Bristol, UK), and ramosetron (kindly supplied by Astellas Pharma, Tokyo, Japan). Indomethacin was suspended in saline with a drop of Tween 80 (Wako, Osaka, Japan). Ramosetron was dissolved in and the other agents were suspended in 0.5%

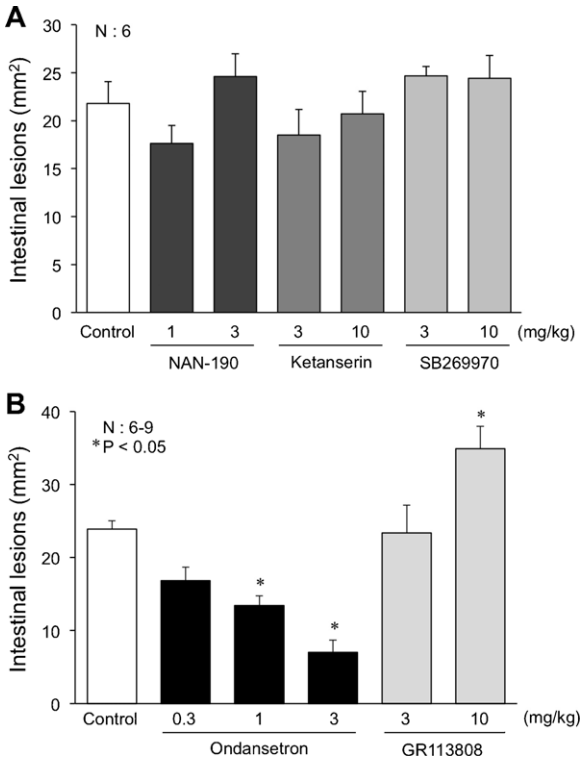

Fig. 2. Effects of various 5-HT receptor antagonists on indomethacin-induced small intestinal lesions in mice. Animals were given indomethacin (10 mg/kg, p.o.) and sacrificed 24 h later. NAN-190 (a 5-HT1 receptor antagonist; 1 and 3 mg/kg), ketanserin (a 5-HT2 receptor antagonist; 3 and 10 mg/kg), SB269970 (a 5-HT7 receptor antagonist; 3 and 10 mg/kg), ondansetron (a 5-HT3 receptor antagonist; 0.3, 1, and 3 mg/kg), and GR113808 (a 5-HT4 receptor antagonist; 3 and 10 mg/kg) were given i.p. twice, 0.5 h before and 8 h after the administration of indomethacin. Data are presented as the mean ± S.E.M. for 6–9 mice. *Significant difference from control (vehicle alone) at $P < 0.05$. (A) effects of NAN-190, ketanserin, and SB269970 and (B) effects of ondansetron and GR113808.