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Neurogenic contraction of mouse rectum via the cyclooxygenase pathway: Changes of PGE_2-induced contraction with dextran sulfate sodium-induced colitis

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ABSTRACT

Recent reports suggest that cyclooxygenases (COXs) including COX-2 are constitutively expressed, and prostaglandins (PGs) regulate motility and/or contraction in the colon and rectum. This study examines the role of COXs in the regulation of neuromuscular function in longitudinal preparations of isolated rectum and distal colon (Side A, close to the transverse colon; and Side B, close to the rectum) in normal mice and after the induction of colitis by dextran sulfate sodium (DSS). In control rectum, electrical stimulation (ES)-induced contractions were inhibited by atropine and by COX inhibitors, in an independent manner. PGE_2 at 3 μM caused a marked contraction, but the secondary response at 20 min after the first application was 60% desensitized. In rectum from DSS-treated mice, spontaneous and ES-induced contractions were significantly less intense than in the control preparations, and the response to PGE_2 was abolished but that to 3 μM acetylcholine was not. In control distal colon, the responses to PGE_2 in neither side were desensitized by the repeated application. In DSS-treated distal colon, PGE_2 response was impaired in the two regions, and was desensitized on Side B more than Side A. DSS treatment impaired contractions by 40 mM KCl in rectum and on Side B but not Side A. DSS treatment increased COX-2 expression in rectum, but not in distal colon. These findings suggest that the induction of colitis by DSS affects ES- and PGE_2-regulated motility in the order rectum > distal colon close to the rectum > distal colon in mice.

1. Introduction

Various factors including cytokines, prostaglandins (PGs), and nitric oxide are known to have physiological and pathophysiological roles in the gastrointestinal (GI) tract including the distal colon and rectum, regulating water transport, blood flow, and motility. Two isoforms of cyclooxygenase (COX) have been recognized, COX-1 (an endogenous type) and COX-2 (an inducible type). In general, PGs produced via COX-2 are involved in both inflammation and regeneration whereas PGs derived from COX-1 exert immunomodulatory, cytoprotective, and proangiogenic effects (for a review, see Ref. [1]). It is reported that COX-1 and COX-2 are expressed in the neuromuscular compartment of normal human and mouse colon, where they modulate the cholinergic excitatory regulation of colonic motility [2]. In the presence of colitis, the expression of COX-2 was up-regulated and seemed to play a predominant role in modulating neuromuscular function in rat colon [3]. Receptors for prostanoids are present on smooth muscle cells, causing contraction or relaxation depending on the receptor subtype [4,5]. Prostanoid receptors can be functionally grouped into three categories. EP1-, TP- and FP-receptors are contractile receptors. EP2-, EP4-, IP- and DP-receptors are relaxant receptors. The EP3-receptor is an inhibitory receptor inhibiting muscle relaxation, although its activation causes the contraction of smooth muscles in several GI tissues [4,5]. In addition, prostanoid receptors exist on cholinergic neurons in the GI tract, where their activation increases or decreases the release of acetylcholine (ACh) depending on the tissue and species. In longitudinal preparations of muscle-myenteric plexus from guinea-pig ileum, treatment with indomethacin inhibited contractions and the release of ACh induced by nicotine, and the inhibitory effects were prevented by PGE_2 and PGI_2 [6,7]. These reports suggest a role for PGs in contractile responses regulated by neuronal- and non-neuronal pathways in GI tissues. Although PGE_2 caused the contraction of longitudinal preparations of rat rectum [8], the precise role of COX and/or PGs in contractile responses in the rectum has not been elucidated.

Abbreviations: PGs, prostaglandins; GI, gastrointestinal; COXs, cyclooxygenases; ACh, acetylcholine; IBD, inflammatory bowel disease; UC, ulcerative colitis; DSS, dextran sulfate sodium; ES, electrical stimulation; TTX, tetrodotoxin; 5-HT, 5-hydroxytryptamine.

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Inflammation of the GI tract, which occurs during inflammatory bowel disease (IBD), acute bacterial enteritis, and nematode-infections, markedly alters GI function [9–11]. Data obtained from patients with IBDs such as Crohn's disease and ulcerative colitis (UC) and animal models of IBD have suggested a role for inflammatory effects on neurons in the symptoms associated with IBD [9,12–14]. One animal model of human colitis involves dextran sulfate sodium (DSS) being administered in drinking water to rodents including mice. This model displays several characteristics of both UC and Crohn's disease, and it has been suggested that Th-1-dominant inflammation is involved in the pathogenesis of acute DSS colitis, while the chronic DSS model is characterized by Th-1 and Th-2 cytokines [15,16]. There is evidence of increased production of prostanoids such as PGE_2 and PGI_2 (prostacyclin) and expression of COXs including COX-2 in the colon of patients with active IBD and mammals with experimental colitis [17–21]. COX-2 plays a key role in IBD-related inflammation through its production of PGs [22,23], although COX-2 and/or PGs are proposed to have suppressive effects on the development and exacerbation of colitis under several conditions [24,25]. However, the exact roles of COXs and PGs in contractile responses in the rectum with and without colitis have not been elucidated.

In general, the rectum is thought to function as a reservoir, and the anal canal is a sphincter area maintaining a high resting tone to resist the inappropriate passage of feces and gas. The internal anal sphincter, which is anatomically well defined as a pronounced thickening of the circular smooth muscle layer separated by a short transition zone, is of great significance in retaining this resting tone [26]. The volume required to induce a desire to defecate and to cause sustained anal relaxation was lower in patients with active UC compared with controls, and circular muscle preparations of UC rectum had increased sensitivity to carbachol [27]. The rectum possesses mechanical passive and active properties, including spontaneous resting tension and dynamic motility, comparable to those of the internal anal sphincter, but limited information is available concerning the contractile response in whole longitudinal preparations of rectum. Hyperalgesia to mechanical distensions of the rectum in patients with active UC is likely as a result of changes in mechanical properties of neurons in the rectum because of edema and sustained contractions of the smooth muscle [28,29], in addition to the inflammation per se. PGE_2-induced contractions in rat rectum [8] and the role of nitric oxide in muscles from human rectum [30,31] were reported. However, the precise mechanism(s) of neuronal regulation for rectal motility and for the changes induced by UC have not been elucidated. In the present study, the contractile response in longitudinal preparations from the rectum to electrical stimulation (ES) and to the stimulation of receptors by ACh and PGE_2, and changes therein in rectum from mice having DSS-induced UC, were investigated.

2. Experimental procedures

2.1. Animals and induction of experimental colitis

Mail ddY mice were purchased from SLC Co. (Shizuoka, Japan). Animals, weighing 34–41 g, in groups of 5 or 6, were used. They were housed under controlled environmental conditions (temperature of $24 \pm 2\,^{\circ}C$ and lights on between 7:00 a.m. and 7:00 p.m.) and fed commercial MF chow (Oriental Yeast Co. Ltd., Tokyo, Japan) for at least 1 week before the experiments. Before the experiments for measuring contractile response, mice were kept individually and fasted for 16–18 h with free access to water. They were sacrificed by cervical dislocation. Colitis was induced by adding DSS (M.W. 5000, Wako, Osaka, Japan) to their drinking water to a final concentration of 2.5% as described previously [32]. Animals with diarrhea and bloody excrement were sacrificed from 7 to 10 days following the DSS treatment, and the decrease of body weight was within 15–25%, but not over 25%, of that before the treatment. Since reactivity to DSS treatment differed slightly between individual animals, we used those animals that met the above criteria. Housing and handling of animals were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society, and the experiments were approved by the Experimental Animal Committee of Chiba University.

2.2. Reagents

The following drugs and chemicals were used: ACh chloride, bethanechol and indomethacin (Sigma, St. Louis, MO, USA); tetrodotoxin (TTX, Wako, Osaka, Japan); atropine sulfate (Nacalai, Kyoto, Japan); PGE_2 and diclofenac (Cayman, Ann Arbor, MI); and arachidonic acid (MP Biomedicals, Solon, OH). PGE_2, indomethacin and diclofenac were dissolved in dimethyl sulfoxide, and the other reagents were dissolved in distilled water prior to dilution in Krebs–Henseleit buffer. The stock solution of arachidonic acid was dissolved in ethanol, and diluted with the buffer containing 0.001% albumin. The vehicle containing dimethyl sulfoxide or ethanol at less than 0.5% had no effect on contraction of the colon and rectum in the presence or absence of stimuli such as ACh and ES.

2.3. Preparations and measurement of contractile response

The entire length of the colon was removed in Krebs–Henseleit buffer (NaCl, 112.0 mM; KCl, 5.9 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.2 mM; NaH_2PO_4, 1.2 mM; $NaHCO_3$, 25.0 mM; glucose, 11.5 mM; pH 7.4). The whole segment, 10 mm in length from the anal orifice, was used as the rectum. The segments (10 mm in length) of the distal (descending) colon were taken 35–55 mm from the ileo-caecal junction (10–30 mm from the anal orifice). In some experiments, the distal colon was separated into two parts; Side A close to the transverse colon and usually referred to as the distal colon, and Side B close to the rectum, and sensitivity to stimuli such as PGE_2 was measured. The preparations (including mucosa, circular layer and neuronal plexus) were usually suspended in the longitudinal direction under a 1-g load in a 5-mL organ bath containing the buffer. Since spontaneous contractions in the longitudinal preparations were much less than those in the circular preparations, we used the longitudinal preparations in the present study. The bath was maintained at $37\,^{\circ}C$ and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. One end of each segment was attached to an isometric transducer (T-7-8-240, Orientic Co., Tokyo, Japan) or to an isotonic transducer (Type 45347, NEC San-ei, Tokyo), and recordings were made with a recorder (056, Hitachi, Mito, Japan) via a DC strain-amplifier (6M92 or AS2102, NEC San-ei, Tokyo, Japan). The other end was mounted on a rigid support or on an anodal electrode placed at the bottom of the bath. The reagents were added to the organ bath. At the start of each experiment, the maximum response to 3 μM ACh was measured in each preparation to evaluate the effects of the reagents tested such as PGE_2. For treatment with the inhibitors or receptor antagonists, the preparations were incubated for the indicated period with the reagents, after which contractile response was measured in the presence of the respective reagents. The inhibitors or antagonists were used at concentrations reported previously [32,33]. A stimulator (Sen-7203, Nihon Koden, Tokyo) was used for the electrical transmural stimulation (ES) of the rectum, and the preparations were stimulated by platinum needle-ring (a ring was placed 20 mm above the base of the needle 5 mm in length) electrodes. The conditions were 10 V, 1 ms duration, 5 ms interval, and 10 trains, and the responses were recorded with a 1-min interval between tests. For contraction