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Mast cells and the cyclooxygenase pathway mediate colonic afferent nerve sensitization in a murine colitis model☆

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ABSTRACT

Introduction: Intestinal inflammation alters colonic afferent nerve sensitivity which may contribute to patients' perception of abdominal discomfort. We aimed to explore whether mast cells and the cyclooxygenase pathway are involved in altered afferent nerve sensitivity during colitis.
Methods: C57Bl6 mice received 3% dextran-sulfate sodium (DSS) in drinking water for 7 days to induce colitis. Control animals received regular water. On day 8 inflammation was assessed in the proximal colon by morphology and histology. Extracellular afferent nerve discharge was recorded from the mesenteric nerve of a 2 cm colonic segment. Subgroups were treated in vitro with the mast cell stabilizer doxantrazole (10^{-4} M) or the cyclooxygenase inhibitor naproxen (10^{-5} M).
Results: DSS colitis resulted in morphological and histological signs of inflammation. At baseline, peak firing was 11 ± 2 imp s^{-1} in colitis segments and 5 ± 1 imp s^{-1} in uninflamed control segments ($p<0.05$; mean ± SEM; each $n = 6$). In colitis segments, afferent nerve discharge to bradykinin (0.5 μM) was increased to 47 ± 7 compared to 23 ± 6 imp s^{-1} in recordings from non-inflamed control tissue ($p<0.05$). Mechanosensitivity during luminal ramp distension (0–80 cm H_2O) was increased reaching 24 ± 5 imp s^{-1} at 80 cm H_2O during colitis compared to 14 ± 2 in non-inflamed controls ($p<0.05$). Doxantrazole or naproxen reduced afferent discharge to bradykinin and luminal ramp distension in colitis segments to control levels.
Conclusion: Intestinal inflammation sensitizes mesenteric afferent nerve fibers to bradykinin and mechanical stimuli. The underlying mechanism responsible for this sensitization seems to involve mast cells and prostaglandins.

1. Introduction

Chronic inflammatory bowel disease (IBD) is characterized by abdominal pain and discomfort for affected patients. These symptoms are initiated by the release of an array of inflammatory mediators with subsequent sensitization of visceral afferent nerves (Bielefeldt et al., 2009). Despite this common knowledge, our understanding of mechanisms that may contribute to this sensitization and subsequent perception of pain during intestinal inflammation is limited.

Visceral sensitivity during intestinal inflammation is most commonly studied in rat or mice by characterization of the viscero-motor response i.e. behavior and/or muscle contraction of the abdominal wall during intestinal distension (Christianson and Gebhart, 2007). This method is indirect and the viscero-motor response as outcome parameter for visceral sensitivity may be modulated at various levels such as the intestinal wall, afferent pathways, the central nervous system (CNS), and efferent pathways (Coutinho and Gebhart, 1999; Friedrich and Gebhart, 2000). Contrary to this indirect and rather unspecific method of studying visceral sensitivity, models of direct recordings from extrinsic afferent nerves innervating the intestine allow to measure peripheral afferent sensitivity directly without potential modulation in the CNS. These direct recordings also allow to test specific chemical or mechanical stimuli and the effect of antagonists interfering with the action of different mediators. Thus, direct recordings from afferent nerves permit to study the potential involvement of different mechanisms specifically (Karpitschka and Kreis, 2010).

Coldwell et al. characterized afferent nerve responses to the mediator 5-hydroxytryptamine (5-HT) and localized mechanical stimulation of the colon to project in the corresponding lumbar splanchnic nerve (Coldwell et al., 2007). They found increased sensitivity to both stimuli during acute dextran sulfate sodium (DSS) induced colitis which

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persisted for 5-HT after the acute inflammation had subsided. Further studies provided evidence that transient receptor potentials of the vanilloid type 1 (TRPV1, De Schepper et al., 2008a, 2008b), type 4 (TRPV4, Sipe et al., 2008), the transient receptor potential channel A1 (Brierley et al., 2009), and protease activated receptor PAR_2 (Sipe et al., 2008) on colonic afferent nerve terminals are involved in afferent sensitization during experimental colitis.

In the present study, our interest was to investigate the role of mast cells and the cyclooxygenase pathway for afferent sensitivity during colitis. We specifically investigated afferent sensitivity to bradykinin (BK) as its release and sensitizing action on afferent nerve terminals is a crucial mechanism leading to the generation of pain in somatic models (Couture et al., 2001). For the same reason the afferent nerve response to mechanical distension was also tested.

We hypothesized that BK- and mechanosensitivity of afferent nerve fibers innervating the colon is increased during DSS induced colitis, and that mast cells and the cyclooxygenase pathway are involved.

2. Methods

2.1. Animals/DSS colitis

Experiments were performed with male C57BL/6 mice (Charles River, Sulzfeld, Germany) weighing approximately 20 g. Animals were held under a 12 h/12 h dark/light cycle with free access to food and water before and during experiments. Animal experiments were approved by the local Institutional Review Board (Regierung von Oberbayern). Colitis was induced by feeding animals 3% dextransulfate sodium (DSS) which was dissolved in the animals' drinking water and given for seven consecutive days as described previously (Okayasu et al., 1990; Krieglstein et al., 2001). Control animals received vehicle only i.e. normal tap water. Animals were sacrificed on day eight after the beginning of DSS or vehicle administration by inhalation of an anesthetic overdose (Isoflurane, Abbott, Baar, Switzerland). Then, after a quick laparotomy, the colon was removed with the adjacent mesentery attached.

2.2. Tissue preparation for afferent nerve recordings

The colon and its mesentery were placed in a culture dish containing ice-cold Kreb's solution and the cecum was cut off. Then, under stereoscopic vision (operating microscope, Wild M3Z, Heerburg, Switzerland), the mesentery was dissected off the colon from distal to proximal except for the first 2 cm of proximal colon i.e. the first part distal to the cecum. These 2 cm of ascending colon with the mesentery attached were prepared for afferent nerve recordings.

2.3. Technique of afferent nerve recordings

The colonic segment was placed into a custom-made organ bath that consisted of two chambers. In a perfusion chamber, the segment was superfused with Kreb's buffer gassed with a O_2/CO_2 mixture (95%/5%; composition of Kreb's (mM): Na^+ 143.5, K^+ 5.9, Cl^- 126, Ca^{2+} 2.5, Mg^{2+} 1.2, H_2PO_4 1.2, SO_4 1.2, HCO_3^- 25, glucose 10 and sodium butyrate 1, pH 7, superfused at a rate of 10 ml min^{-1}, temperature 32 °C). In order to eliminate spontaneous colonic motility that rendered afferent nerve recordings impossible in pilot experiments, the L-type calcium channel blocker nifedipine was added to the perfusion solution at a concentration of 1 μM as described by others (Hicks et al., 2002). Continuous superfusion with Kreb's in the perfusion chamber was ensured with the help of a pump (Minipuls 3, Gilson, France).

The mesenteric arcade next to the colonic segment was guided through an opening into a recording chamber. The opening was sealed with Vaseline before the recording chamber was filled with colorless heavy liquid paraffin (32 °C) for insulation. Both ends of the colonic segment were cannulated and tied. The lumen was continuously perfused with Kreb's from the proximal side of the colon (10 ml per hour), while the distal cannula remained open to the atmosphere during the experiment unless mechanical ramp distension was performed (see below). Intraluminal pressure was monitored continuously by a separate channel in the proximal cannula which was connected to a pressure transducer (Neurolog pressure amplifier NL 108, Digitimer Ltd., Welwyn Garden City, UK). The pressure at baseline was typically 0.5–2 cm H_2O.

In the recording chamber, the mesenteric nerve bundle was dissected out of the mesentery attached to the colonic segment. The mesenteric nerve was then attached to one arm of a bipolar platinum recording electrode, with a fiber of connective tissue wrapped around the other electrode serving as a reference. The electrodes were connected to a single channel 1902 preamplifier/filter (Cambridge Electronic Design (CED), Cambridge, UK), and the signal was amplified 10.000 times and filtered with a bandwidth of 100 Hz to 1 kHz. Signals from the pressure transducer recording the intraluminal intestinal pressure were relayed to another single channel 1902 preamplifier/filter. The output from the preamplifier/filter and the signals from the pressure transducers were transferred to a power Micro 1401 interface system (CED) saved and viewed online by running Spike 2 software (version 4.01; CED).

2.4. Experimental protocol

Once a stable recording from the mesenteric colonic nerve was established for 15 min, spontaneous afferent nerve discharge was recorded at baseline for 10 min. Then, mechanical stimulation of the colonic segment was performed by ramp distension to 80 cm H_2O in approximately 120 s. For this aim, the outlet of the cannula in the lumen of the intestinal segment was clamped and perfusion with Kreb's solution was continued (10 ml per hour). Thereafter, the distal outlet was reopened and a minimum of 10 min was allowed for afferent discharge to return to baseline levels before the perfusion was stopped and BK was administered into the organ bath. BK was added in a volume of 250 μl from a stock solution of 10 μM for BK resulting in a final concentration of 0.5 μM in the perfusion chamber. This dose was determined as an intermediate dose in preliminary dose–response experiments (Fig. 1). After 2 min perfusion was started again in order to wash out BK from the organ bath.

Colonic segments were studied from 4 experimental groups (each n = 6, one segment was harvested from one animal):

1. Vehicle pretreated control mice.
2. Mice with DSS colitis.
3. Mice with DSS colitis and administration of the mast cell inhibitor doxantrazole (10^{-4} M) in the organ bath to investigate effects of mast cell mediator release on afferent nerve discharge during colitis.
4. Mice with DSS colitis and administration of the cyclooxygenase inhibitor naproxen (10^{-5} M) in the organ bath to investigate effects

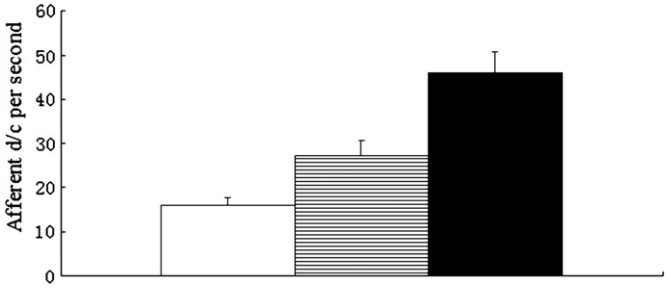

Fig. 1. Dose–response relationship for mesenteric afferent nerve discharge to rising doses of serosal bradykinin. Note that the 0.5 μM dose is neither a minimal nor a submaximal dose as regards the response in afferent nerve discharge. d/c: discharge; white bar: 0.25 μM, bar with horizontal stripes: 0.5 μM, black bar: 1 μM. Data are mean ± SEM (n = 6).