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The early effect of dextran sodium sulfate administration on carbachol-induced short-circuit current in distal and proximal colon during colitis development

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Short title

Effect of DSS on carbachol-induced current in murine colon

Summary

Increased colonic Cl^- secretion was supposed to be a causative factor of diarrhea in inflammatory bowel diseases. Surprisingly, hyporesponsiveness to Cl^- secretagogues was later described in inflamed colon. Our aim was to evaluate changes in secretory responses to cholinergic agonist carbachol in distal and proximal colon during colitis development, regarding secretory activity of enteric nervous system (ENS) and prostaglandins. Increased responsiveness to carbachol was observed in both distal and proximal colon after 3 days of 2% dextran sodium sulfate (DSS) administration. It was measured in the presence of mucosal Ba^{2+} to emphasize Cl^- secretion. The described increase was abolished by combined inhibitory effect of tetrodotoxin (TTX) and indomethacin. Indomethacin also significantly reduced TTX-sensitive current. On the 7th day of colitis development responsiveness to carbachol decreased in distal colon (compared to untreated mice), but did not change in proximal colon. TTX-sensitive current did not change during colitis development, but indomethacin-sensitive current was significantly increased the 7th day. Decreased and deformed current responses to serosal Ba^{2+} were observed during colitis induction, but only in proximal colon. We conclude that besides inhibitory effect of DSS on distal colon responsiveness, there is an early stimulatory effect that manifests in both distal and proximal colon.

Key words

cholinergic; ion transport; colitis; distal and proximal colon; Ussing chamber

Introduction

Ulcerative colitis (UC) and Crohn's disease are chronic inflammatory bowel diseases (IBD) in which the role of interactions between genetic, immunologic, microbial and environmental factors is expected, but exact etiology and pathogenesis still remain unclear (Kaser et al. 2010). A number of animal models mimicking different aspects of IBD were developed (Westbrook et al. 2010). In mice, an experimental colitis is probably the most commonly induced by oral administration of dextran sodium sulfate (DSS) (Okayasu et al. 1990). DSS induces acute colitis with symptomatic and histopathologic findings such as rectal bleeding, body weight loss, shortening of the colon, distortion of crypts, loss of goblet cells and infiltration of leukocytes. It is accompanied by increased transcription levels of inducible forms of cyclooxygenase (COX-2) and NO synthase (iNOS), and proinflammatory cytokines including tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) (Yan et al. 2009, Gravaghi et al. 2011).

Inflammation strongly affects the intestine including its motility, activity of gut nervous system and intestinal transport. It is associated with increased ion and water secretion, resulting in a diarrhea, a typical life-complicating symptom of IBD that affects virtually 100% of UC patients. The overstimulation of colonic Cl⁻ secretion followed by water secretion was suspected as a causative factor of diarrhea because secretory effects of proinflammatory mediators TNF- α , IL-1 β or prostaglandins were reported *in vitro* (Bode et al. 1998). However, studies of the intestine in IBD patients and different animal models revealed a reduced sensitivity to secretagogues, including mediators of enteric nervous system (ENS), prostaglandins and cytokines (Martínez-Augustin et al. 2009).

An altered response of inflamed colon to secretory stimuli was demonstrated also for ENS mediator acetylcholine, respectively its stable analog carbachol (Diaz-Granados et al.

2000). Carbachol triggers Cl^- secretion in colonocytes by activation of Ca^{2+} dependent pathway via muscarinic receptors (Haberberger et al. 2006, Hirota and McKay 2006a). An increase in concentration of intracellular Ca^{2+} results in basolateral K^+ channel opening ($\text{K}_{\text{Ca}3.1}$) and membrane hyperpolarization, which drives the apical Cl^- efflux (Flores et al. 2007, Matos et al. 2007). The extent of carbachol-sensitive Cl^- secretion depends on (i) the resting membrane potential (respectively, the difference between membrane potentials before and after K^+ channels opening), (ii) the intracellular concentration of Cl^- , which is transported into the cell by $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter isoform 1 (NKCC1) localized on the basolateral membrane and (iii) the activity of cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated Cl^- (HCO_3^-) ion channel expressed on the apical membrane (Hirota and McKay 2006b). To function properly, the Ca^{2+} -dependent signaling pathway seems to require a baseline level of cAMP because it stimulates NKCC1 and Na^+/K^+ ATPase activities and opens CFTR channel (Barret and Keely 2000). The most important modulators of cAMP levels in enterocytes are likely the VIPergic part of ENS tonus and prostaglandins (Kunzelmann and Mall 2002).

Studies of colonic secretion during acute colitis focus almost exclusively on an inflamed tissue several days after DSS insult (Asfaha et al. 1999, Diaz-Granados et al. 2000, Sayer et al. 2002, Green et al. 2004, Perez-Navarro et al. 2005), but changes of intestinal secretion during the development of acute colitis have not been investigated. Nevertheless, DSS administration may influence intestinal secretion much earlier than considered before. Johansson et al. (2010) demonstrated that 3% DSS can decrease thickness of protective mucus layer within 15 min and increase permeability of mucus layer to bacteria already after 12 hours. Therefore, our aim was to characterize the effect of DSS administration on carbachol-induced currents in distal and proximal colon during the colitis development, regarding secretory activity of ENS and prostaglandins.

Methods

Animals. Male BALB/c mice (10-14 weeks old, Institute of Physiology, Czech Academy of Sciences, Prague) were housed at controlled temperature (21°C) and photoperiod (12:12-hr light-dark cycle) with standard mouse chow and tap water ad libitum (according to guidelines of the European Community). Mice were allowed to acclimate for at least a week before experiments or induction of colitis. The study was approved by the Institutional Animal Care Committee.

Colitis induction. Mice were given 2% (w/v) DSS (MW 40 kDa; Affymetrix UK Ltd., United Kingdom) in drinking water for 7 days. Untreated mice (day 0), mice during DSS drinking (day 3, 5 and 7) and mice 4 weeks after DSS removal (day 35) were killed by decapitation under ether anesthesia. The entire colon was excised, rinsed to remove its content, opened along the mesenteric border and used for further experiments, or immediately frozen in liquid nitrogen for RT-PCR.

Colitis assessment and histological evaluation. The severity of colitis was assessed on days 0, 3, 5, 7 and 35 using macroscopic markers (rectal bleeding, body weight and colon length), the transcript levels of proinflammatory markers TNF- α , IL-1 β and COX-2 (constitutive COX-1 measured as well) and histological scoring. The parts of distal colon corresponding to segments utilized for electrophysiological and mRNA analysis were fixed in 8% formaldehyde, paraffin embedded, sectioned and stained with hematoxylin and eosin. Blind sections were scored by a pathologist (activity of inflammation, 0-3; number and size of

lymphoid follicles, 0-3; and crypt distortion extent, 0-3) and the total score was calculated for each colon sample.

Ussing chamber experiments. Whole-thickness segments of distal and proximal colon were mounted in a modified Ussing chambers (0.096 cm² opening). The tissue was bathed on both sides in Krebs-Ringer solution (composition in mM: 140.5 Na⁺, 5.4 K⁺, 1.2 Ca²⁺, 1.2 Mg²⁺, 119 Cl⁻, 21 HCO₃⁻, 0.6 H₂PO₄⁻, 2.4 HPO₄²⁻, 10 D-mannitol, 10 D-glucose, 2.5 L-glutamine, 0.5 β-hydroxybutyrate) gassed with carbogen (95% O₂ + 5% CO₂) and kept at 37 °C. After an equilibration period of 30 min (20 min in open circuit mode and 10 min in voltage-clamp mode), the response to serosal carbachol (10⁻⁴ M), serosal TTX (10⁻⁶ M), serosal indomethacin (5.10⁻⁵ M), and mucosal and serosal Ba²⁺ (5.10⁻³ M) was recorded by a computer-controlled voltage clamp (Müssler Scientific Instruments, Aachen, Germany). Net active ion transport across the epithelium was measured as a short-circuit current (SCC; expressed as μA.cm⁻²) in voltage-clamp mode. In experiments, where a combination of several drugs was used, they were added sequentially in 5-min intervals except of indomethacin, where 10-min interval was used because of a slower colonic tissue response to this drug. In addition to SCC, the potential difference and tissue resistance were recorded at sampling frequency 1 Hz, the data stored and further processed. To avoid underestimating electrogenic Na⁺ absorption via epithelial Na⁺ channel (ENaC), responses to amiloride (10⁻⁵ M) were measured on untreated distal and proximal colon, during and after DSS administration. No amiloride-sensitive current was detected (data not shown).

RNA extraction, reverse transcription and quantitative real-time PCR. Total RNA was isolated from whole-thickness segments of distal and proximal colon using GenElute Total Mammalian RNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA). The RNA

concentration was estimated spectrophotometrically at 260 nm using Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). The first strand cDNA was synthesized using 1 µg of RNA with random hexamers (0.5 µg) in a reaction volume of 15 µl using Im-Prom II Reverse Transcription System (Promega, Madison, WI, USA) according to manufacturer's protocol. To detect relative abundance of mRNA of particular genes, real-time PCR was performed using ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) in 20 µl reaction volume. Each reaction contained Gene Expression Master Mix, gene-specific pre-made FAM-labeled TaqMan probes and primers or VIC-labeled TaqMan for normalization gene (TaqMan Gene Expression Assays, Applied Biosystems) and 1 µl of 4 times diluted cDNA. Following thermal conditions were applied: 2 min at 50°C, initial denaturation step for 10 min at 95°C followed by 45 cycles of denaturation for 15 s at 95°C and annealing and elongation for 1 min at 60°C. Fluorescence acquisition occurred at the end of each elongation step. The assays IDs and accession numbers (NCBI RefSeq) of related genes are as follows: TNF- α (Mm00443258_m1, NM_013693.2), IL-1 β (Mm00434228_m1, NM_008361.3), COX-1 (Mm00477214_m1, NM_008969.3), COX-2 (Mm00478374_m1, NM_011198.3). Relative quantification was assessed from obtained Ct values (7000 System SDS Software, Applied Biosystems) using standard curve method. The data were normalized to 18S rRNA (4308329, NR_003278). Ct values of the normalization gene were consistent and did not change across experimental groups. To get a clear demonstration, the mean value of distal colon controls was arbitrarily set at 1 for each gene.

Statistical analysis. Non-electrophysiological data were compared by one-way analysis of variance (ANOVA) combined with Dunnet's *post hoc* test and the values are reported as means \pm SEM. Baseline SCC and tissue resistance were compared by Student's t-test or one-

way ANOVA combined with Dunnet's *post hoc*. Results are expressed as means \pm SEM. However, induced current responses were not compared by standard method, i.e. maximal deviations from baseline SCC compared by Student's t-test or one-way ANOVA. A new approach for comparison of two responses was used, taking into account not only the maximal deviations, but also differences in the shape of the responses. This approach was chosen since it was impossible, using the standard method, to statistically distinguish some responses that were evidently different in shape, but had similar maximal deviations. For example, this was the case for comparison of carbachol-induced currents (in the presence of mucosal Ba²⁺) in distal and proximal colon of untreated mice (Results; Fig. 3). Because of that new method, SCC records were further processed before statistical evaluation. All 5-min parts of record (or 10-min for indomethacin) corresponding to the specific drug combination were extracted from original records and aligned on an average curve. Each point of the average curve represents mean \pm SEM. Only 270 sec of aligned records (or 540 sec for indomethacin) that corresponded to the specific drug combination were used for subsequent analysis. Maximal or minimal deviation from baseline SCC that is used in the text where graphical representation is not possible, is presented as means \pm SEM. Notation Δ SCC₂₇₀ (or Δ SCC₅₄₀ for indomethacin) is used to emphasize that maximum or minimum value was found on 270 sec long (or 540 sec for indomethacin) average curve. A repeated measures ANOVA was used to statistically compare two average curves. Area under the curve was used as a dependent variable for all comparisons. Strictly speaking, the area under each aligned corresponding record was parted into 9 areas (or 18 areas for indomethacin), one area corresponding to 30 sec. Each area was calculated as a sum of charges transferred per sec. Number of areas per curve represents a compromise between number of parameters describing a curve and accuracy of the method. The described procedure was repeated with all drug combinations applied to distal or proximal colon. The interaction between different drug combinations (in distal and proximal

colon) and 9 (or 18) parameters describing area changing in time was tested. Because sphericity assumption (Maulchy 1940) was violated, probably due to the character of the data, Hyun-Feldt correction for violations of sphericity was applied when significance calculated. In all comparisons $p \leq 0.05$ was accepted as a statistically significant difference.

Results

Development of DSS-induced colitis

After 2% (w/v) DSS administration for 7 days a majority of mice developed severe colitis. The histological evidence of colitis is given in Fig. 1. The mice lost 20% of their body weight ($n = 16$, $p < 0.001$) and their colons shortened from 9.3 ± 0.5 cm ($n = 4$) to 6.3 ± 0.2 cm ($n = 16$; $p < 0.001$). Traces of rectal bleeding and watery stool were found around rectum. These symptoms were accompanied by significantly increased transcription levels of TNF- α , IL-1 β and COX-2 in distal colon and, with exception of TNF- α , also in proximal colon; although not so markedly (Fig. 2). Surprisingly, COX-1 transcription level was also increased in both distal and proximal colon (Fig. 2). Transepithelial resistance, which is often used as an electrophysiological marker of epithelial disruption, did not decrease in either distal or proximal colon during colitis development. Contrarily, it was significantly increased compared to untreated tissue on the 3rd and the 5th day of DSS administration, but only in the distal colon (day 0: $50.4 \pm 1.0 \Omega \cdot \text{cm}^2$, $n = 97$; day 3: $61.8 \pm 2.1 \Omega \cdot \text{cm}^2$, $n = 34$, $p < 0.001$; day 5: $57.4 \pm 1.6 \Omega \cdot \text{cm}^2$, $n = 31$, $p < 0.01$). The untreated distal and proximal colon did not differ in their transepithelial resistance.

Tonic activity of ENS and prostaglandins during colitis development

In untreated mice, the baseline SCC was significantly higher in proximal colon compared to distal colon (PC: $94.0 \pm 2.9 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 103$; DC: $76.4 \pm 2.7 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 97$, $p < 0.001$). Tonus of ENS, measured as TTX-sensitive current, was similar in distal ($\Delta\text{SCC}_{270} = -19.1 \pm 2.6 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 30$) and proximal colon ($\Delta\text{SCC}_{270} = -20.6 \pm 2.4 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 29$). Likewise, tonic activity of COX-derived mediators, inhibited by indomethacin, was not significantly different in distal ($\Delta\text{SCC}_{540} = -9.6 \pm 2.7 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 16$) and proximal colon ($\Delta\text{SCC}_{540} = -8.2 \pm 1.6 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 16$). During DSS administration, baseline SCC decreased only the 7th day of colitis development and only in distal colon (day 0: $76.4 \pm 2.7 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 97$ vs. day 7: $63.5 \pm 3.8 \mu\text{A}\cdot\text{cm}^{-2}$, $n = 34$, $p = 0.05$). Nevertheless, tonic activity of ENS was stable in both distal and proximal colon. Tonic secretory activity mediated by prostaglandins did not change until the 7th day of colitis development. In DSS treated mice, SCC response to indomethacin approximately doubled compared to untreated tissue in both distal ($p < 0.05$) and proximal colon ($p < 0.001$) (Fig. 3). An interaction between TTX and indomethacin was present throughout all time points measured in distal and proximal colon. Indomethacin reduced TTX-sensitive currents of distal and proximal colon similarly in untreated mice, during DSS administration and 4 weeks after DSS administration. The strongest interaction were found the 7th day of DSS administration (Fig. 3). In the presence of indomethacin TTX-sensitive current was significantly reduced in both distal ($p < 0.01$) and proximal colon ($p < 0.05$), but concurrently, TTX reduced indomethacin-sensitive current in both distal ($p < 0.05$) and proximal colon ($p < 0.001$). In contrast to the inhibitory effect of indomethacin on TTX-sensitive currents, TTX reduced indomethacin-sensitive currents only the mentioned 7th day of colitis development.

Carbachol-induced currents during colitis induction

The response to carbachol was measured in the presence of mucosal Ba^{2+} . It was used to emphasize Cl^- secretion. K^+ secretion accompanies Cl^- secretion and restrains corresponding changes of SCC. A significant difference between carbachol-induced currents in distal and proximal colon was found in untreated mice. The response of distal colon to carbachol was lower and had a shape different from the response of proximal colon ($p < 0.001$; Fig. 4). An early and very strong stimulatory effect of DSS on carbachol-induced current was revealed the 3rd day of colitis development in both colonic segments (Fig. 4). In distal colon, the increased responsiveness to carbachol observed the 3rd day of colitis development declined gradually until the 7th day where it was significantly depressed compared to untreated tissue, and returned to the initial level 4 weeks after the DSS insult (Fig. 4). Slightly different course of responsiveness to carbachol was found in proximal colon. Carbachol induced significantly higher response the 3rd and the 5th day of colitis development, but the responsiveness returned to its initial level already the 7th day and remained unchanged 4 weeks after the DSS insult (Fig. 4). Depressed carbachol-induced current was not observed in proximal colon.

Effect of ENS and prostaglandins during colitis development

In contrast to differences between carbachol-induced currents in the presence of mucosal Ba^{2+} alone, the addition of TTX eliminated these differences, and carbachol-induced currents in distal and proximal colon were found to be identical in untreated mice. TTX significantly decreased responses to carbachol in both distal ($p < 0.01$) and proximal colon ($p < 0.05$). In comparison to the response in the presence of Ba^{2+} and TTX, the addition of indomethacin significantly increased response of proximal colon to carbachol ($p < 0.05$). The response of distal colon was not influenced (Fig. 4). During colitis development, TTX reduced the responsiveness to carbachol significantly the 3rd ($p < 0.01$), the 5th ($p < 0.05$) and the 7th ($p < 0.01$) day in distal colon and the 3rd ($p < 0.05$) and the 5th ($p < 0.001$) day in proximal colon

(Fig. 4). The additive inhibitory effect of indomethacin on TTX-resistant carbachol-induced current was observed the 3rd day of colitis development ($p < 0.001$) and 4 weeks after DSS insult ($p < 0.001$) in distal colon, and the 7th ($p < 0.05$) day of colitis development in proximal colon. The combined effect of TTX and indomethacin on carbachol-induced current in the presence of mucosal Ba^{2+} is displayed on Fig. 4 (last column). It shows the evident increase of inhibitor-sensitive component of carbachol-induced current the 3rd day of colitis development in distal colon, and the 3rd and the 5th day in proximal colon.

Potassium channels involvement

Adding Ba^{2+} to the mucosal side of both distal and proximal colon caused an increase in SCC that likely corresponded to decreased K^+ secretion. Significantly different responses of distal and proximal colon were measured in untreated mice ($p < 0.001$; Fig. 5). Although there are some significant differences throughout colitis development, there is not dramatic change of Ba^{2+} -sensitive currents in either size or shape. A different situation was observed after Ba^{2+} addition to the serosal side of proximal colon during colitis development. Responses to serosal Ba^{2+} could at least partially reflect baseline ion transport activity, but also certainly reflect activity and/or expression of K^+ channels on apical and basolateral membrane. Significantly different responses of distal and proximal colon were measured in untreated mice ($p < 0.001$; Fig. 5). In distal colon, colitis intensified negative response to serosal Ba^{2+} , but only in the 3rd day of colitis development. In the proximal colon, the shape of responses to serosal Ba^{2+} changed dramatically during colitis development and was significantly different compared to untreated mice on the 3rd, the 5th and the 7th day (Fig. 5). These changes were reversible, because 4 weeks after the DSS insult the response to serosal Ba^{2+} was similar to that of untreated tissue (data not shown).

Discussion

DSS administration is the most common murine model of experimental colitis with symptoms that mimic ulcerative colitis (Okayasu et al. 1990). In studies, where decreased responsiveness of distal colon to carbachol was found, 4% DSS was administered for 5 days (Diaz-Granados et al. 2000, Sayer et al. 2002, Green et al. 2004). We used a lower dose of DSS (2%) to slow colitis development and to avoid the increased mortality of BALB/c mice we observed in preliminary experiments. Although increased transcription levels of TNF- α and IL-1 β were observed after DSS treatment also in proximal colon (Azuma et al. 2008, Yan et al. 2009), only the distal or mid-distal colon responses to carbachol were studied (Diaz-Granados et al. 2000, Sayer et al. 2002, Green et al. 2004). Our data confirm increased transcription levels of IL-1 β , but not TNF- α after 7 days DSS administration in proximal colon. Similarly, an upregulation of COX-2 transcription, but no change of COX-1 transcription was described in distal colon during 3% (w/v) DSS colitis induction (Tanaka et al. 2008). We have no explanation for our conflicting data concerning COX-1 expression in distal and proximal colon, except the different mice strain (ICR) used by Tanaka et al. (2008). We suggest that infiltration of immune cells could be involved. Nevertheless, as we used the whole-thickness segments of colon, we cannot specify the source of measured mRNA. Although the transcription of selected molecular markers of inflammation was significantly increased mainly the 7th day of colitis development, we observed an effect of DSS on carbachol-induced current already on the 3rd day. The early effect of DSS is consistent with the fast DSS effect observed in murine colon by Johansson et al. (2010). They recently demonstrated 53% reduction of the mucus layer thickness after 15 min exposure to 3% DSS. In addition, the inner mucus layer that forms a protective barrier against bacteria and is usually devoid of them, was severely impaired by DSS. Already 12 h after DSS exposure large number of bacteria penetrated into that layer (Johansson et al. 2010). Finally, bacterial penetration into

the submucosa, accompanied by leukocytes infiltration, was observed after 5 days of DSS administration. The early changes in colonic secretion that we observed may be at least in part attributable to enterochromaffin cells. These cells are stimulated by penetrating bacteria and secrete serotonin. There is evidence that serotonin availability increases in experimental colitis in murine distal colon (Bertrand et al. 2010). An important role of serotonin was demonstrated by fluoxetine, a selective serotonin reuptake inhibitor that attenuated the severity of DSS-induced acute colitis in mice (Koh et al. 2011). In our experiments, the reduction of increased responsiveness of distal and proximal colon to carbachol by inhibition of ENS activity and prostaglandins synthesis may occur in conjunction with the secretory effect of serotonin. Serotonin modulates intestinal secretion directly or indirectly via ENS and prostaglandins release (Gershon and Tack 2007). But, unchanged TTX-sensitive and indomethacin-sensitive currents on the 3rd and 5th day of DSS administration may contradict it. Inhibitory effect of indomethacin on TTX-sensitive current may be due to the inhibition of direct effect of prostaglandin E₂ on VIP non-cholinergic secretomotor neurons of submucosal plexus, an interaction described on guinea pig ileum (Dekkers et al. 1997). Similarly, inhibitory effect of TTX on the 7th day of DSS administration may be related to this effect and becoming visible after an increase in prostaglandins level. We suggest that prostaglandins level was increased because of the complementary effects of augmented cyclooxygenases expression and IL-1 β expression. IL-1 β was shown to increase prostaglandin E₂ level by Bode et al. (1998). The role of ENS and prostaglandins in maintaining responsiveness of colonocytes to carbachol was shown in mice by Carew and Thorn (2000). Besides the early effect of DSS on carbachol-induced current in distal and proximal colon, we observed an unchanged response to carbachol after 7 days of DSS administration in proximal colon. It contrasts with depressed carbachol-induced current observed in distal colon also by others (Diaz-Granados et al. 2000, Sayer et al. 2002, Green et al. 2004).

Our findings with K^+ channels blocker Ba^{2+} indicate that these channels play a role in intestinal fluid losses in secretory diarrhea. The only K^+ channel involved in K^+ secretion seems to be Ca^{2+} dependent K^+ channel ($K_{Ca1.1}$) that is expressed on both apical and basolateral membrane of colonocytes in both distal and proximal colon. It is sensitive to Ba^{2+} , tetraethylammonium (TEA) or iberiotoxin (Sandle and Hunter 2010, Sorensen et al. 2010). The involvement of $K_{Ca1.1}$ channel is also suggested by increased expression of the channel found in the inflamed human sigmoid and ascending colon of UC patients (Sandle et al. 2007). However, there are no available data on expression of $K_{Ca1.1}$ or other K^+ channels during colitis development that may help explain surprising response of proximal colon to serosal Ba^{2+} during DSS administration. Hirota and McKay (2009) described unchanged expression of basolateral $K_{Ca3.1}$ channel in colonic crypts at either the mRNA or protein level, but only after DSS insult, not during DSS administration. Sandle and Hunter (2010) formulated hypothesis that prostaglandin E_2 could inhibit opening of $K_{Ca1.1}$ channels in the apical membrane through protein kinase A (PKA). It is consistent with our data obtained on untreated mice. Responses of proximal colon to serosal Ba^{2+} in the presence of mucosal Ba^{2+} were similar in shape to the responses to serosal Ba^{2+} during DSS administration (data not shown), but it has to be confirmed by further experiments.

In conclusion, 2% DSS administration affects not only distal, but also proximal colon. Whereas the cholinergic response of acutely inflamed distal colon decreased, there was not any decrease in responsiveness of proximal colon. In contrast, early induction of colitis significantly stimulated carbachol-induced current in both distal and proximal colon after 3 days of DSS administration. This increased capacity of carbachol to induce SSC changes utilizes both ENS (TTX-sensitive) and prostaglandins pathways. Our study provides new insight into the pathophysiology of DSS model of colitis.

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Abbreviations

CFTR	cystic fibrosis transmembrane conductance regulator
COX-1, COX-2	cyclooxygenases type 1, 2
DSS	dextran sodium sulfate
ENaC	epithelial sodium channel
ENS	enteric nervous system
IBD	inflammatory bowel disease
IL-1 β	interleukin-1 β
K _{Ca} 1.1	Ca ²⁺ dependent K ⁺ channel (alternative names BK, KCNMA1)
K _{Ca} 3.1	Ca ²⁺ dependent K ⁺ channel (alternative names IK, KCNN4)
NKCC1	Na ⁺ -K ⁺ -2Cl ⁻ cotransport isoform 1
SCC	short-circuit current
TNF- α	tumor necrosis factor- α
TTX	tetrodotoxin
UC	ulcerative colitis
VIP	vasoactive intestinal peptide

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FIGURE CAPTIONS

Figure 1: Histological evidence of colitis. Samples of distal colon were taken before (day 0), during (day 3, 5 and 7) and 4 weeks after administration of 2% DSS (day 35). Tissue was stained with hematoxylin and eosin, samples were scored as mentioned in Methods and effect of DSS tested by ANOVA followed by Dunnet's *post hoc* test. Significant effect of DSS was found the 7th day of DSS administration and 4 weeks after DSS insult (histological score - day 0: 1.5 ± 0.25 ; day 7: 7.0 ± 0.6 , $p < 0.001$; day 35: 5.0 ± 1.5 , $p < 0.05$; $n = 4$).

Figure 2: Molecular markers of inflammation. Transcription levels of TNF- α , IL-1 β , COX-1 and COX-2 were measured before (day 0), during (day 3, 5 and 7) and 4 weeks after administration of 2% DSS (day 35) in distal (black columns) and proximal colon (white columns). The levels of gene transcription in distal colon corresponding to day 0 were arbitrarily set to 1 for each gene. Significance tested by ANOVA followed by Dunnet's *post hoc* test (compared to day 0: * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$; $n = 4-6$).

Figure 3: Interaction between enteric nervous system (ENS) and prostaglandins. Responses of distal (DC; ●) and proximal colon (PC; ○) to tetrodotoxin (TTX; 10^{-6} M) and indomethacin ($5 \cdot 10^{-5}$ M) the 7th day of 2% DSS administration are displayed. TTX-sensitive current represents tonus of ENS and indomethacin was used to inhibit synthesis of COX-derived mediators. The average curve represents 270 sec tracing (or 540 sec for indomethacin), but only each 10th point (or 20th for indomethacin) is displayed for simplicity. TTX-sensitive current was significantly lower in the presence of indomethacin in both distal ($p < 0.01$) and proximal colon ($p < 0.05$). Similarly, indomethacin-sensitive current was

reduced by TTX in distal ($p < 0.05$) and proximal colon ($p < 0.001$). The number of measured animals is in parentheses.

Figure 4: Effects of enteric nervous system (ENS) and prostaglandins on carbachol-induced current. Mucosal Ba^{2+} (5.10^{-3} M) was used to decrease K^+ secretion and was applied 5 min before carbachol application in all experiments. Response to carbachol (10^{-4} M) alone and in the presence of tetrodotoxin (TTX; 10^{-6} M) or combination of TTX and indomethacin (INDO; 5.10^{-5}) was measured before (day 0), during (day 3, 5 and 7) and 4 weeks after administration of 2% DSS (day 35) in distal (DC; ●) and proximal colon (PC; ○). TTX was used as an inhibitor of ENS and indomethacin inhibited synthesis of COX-derived mediators. The average curve represents 270 sec tracing, but only each 10^{th} point is displayed for simplicity. Combined effect of TTX and indomethacin represents inhibitors-sensitive component of carbachol-induced current. Significant differences between untreated mice (day 0) and mice in various stage of colitis or between tissues in the presence or absence of TTX and indomethacin were tested by repeated measures ANOVA. The number of measured animals is in parentheses.

Figure 5: Ba^{2+} -sensitive currents. Ba^{2+} -sensitive current was measured before (day 0), during (day 3, 5 and 7) and 4 weeks after administration of 2% DSS (day 35, not shown) in distal (DC; ●) and proximal colon (PC; ○). Ba^{2+} was added to mucosal or serosal side. The average curve represents 270 sec tracing, but only each 10^{th} point is displayed for simplicity. Significant differences between untreated mice (day 0) and mice in various stage of colitis were tested by repeated measures ANOVA. The number of measured animals is in parentheses.

FIGURES

Fig. 1.

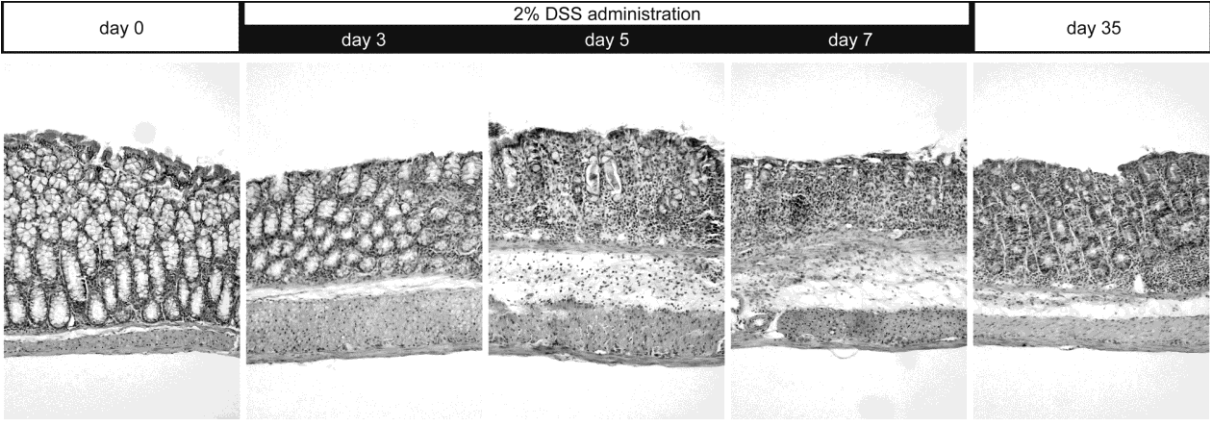


Fig. 2.

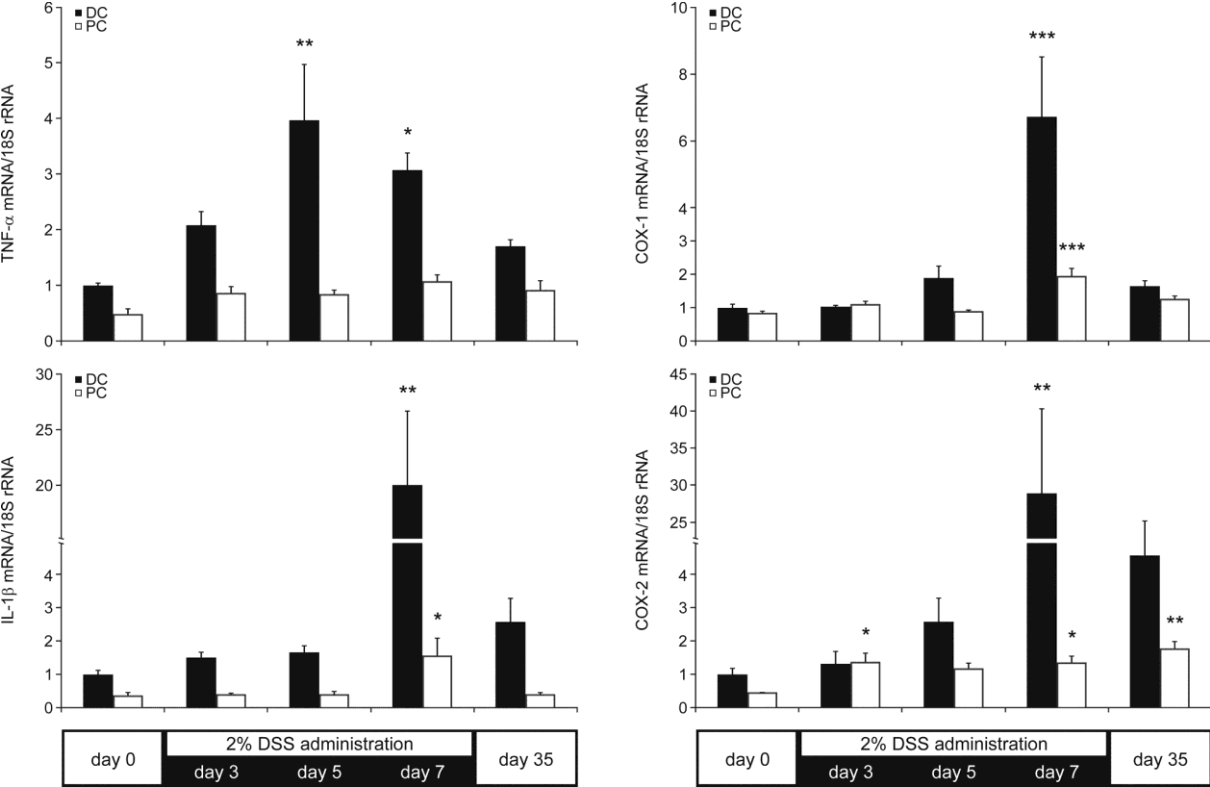


Fig. 3.

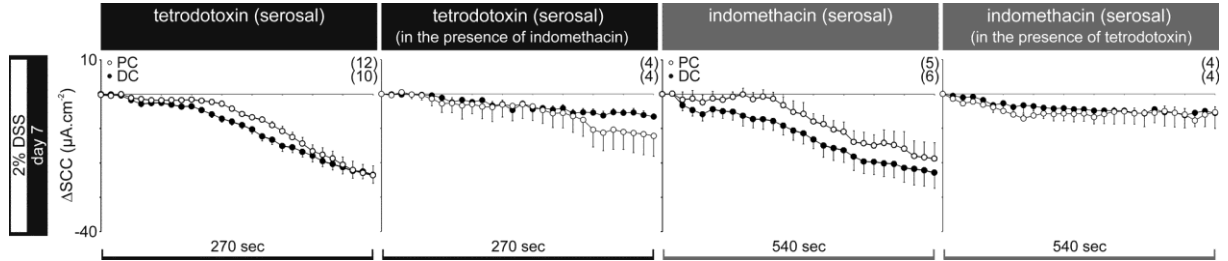


Fig. 4.

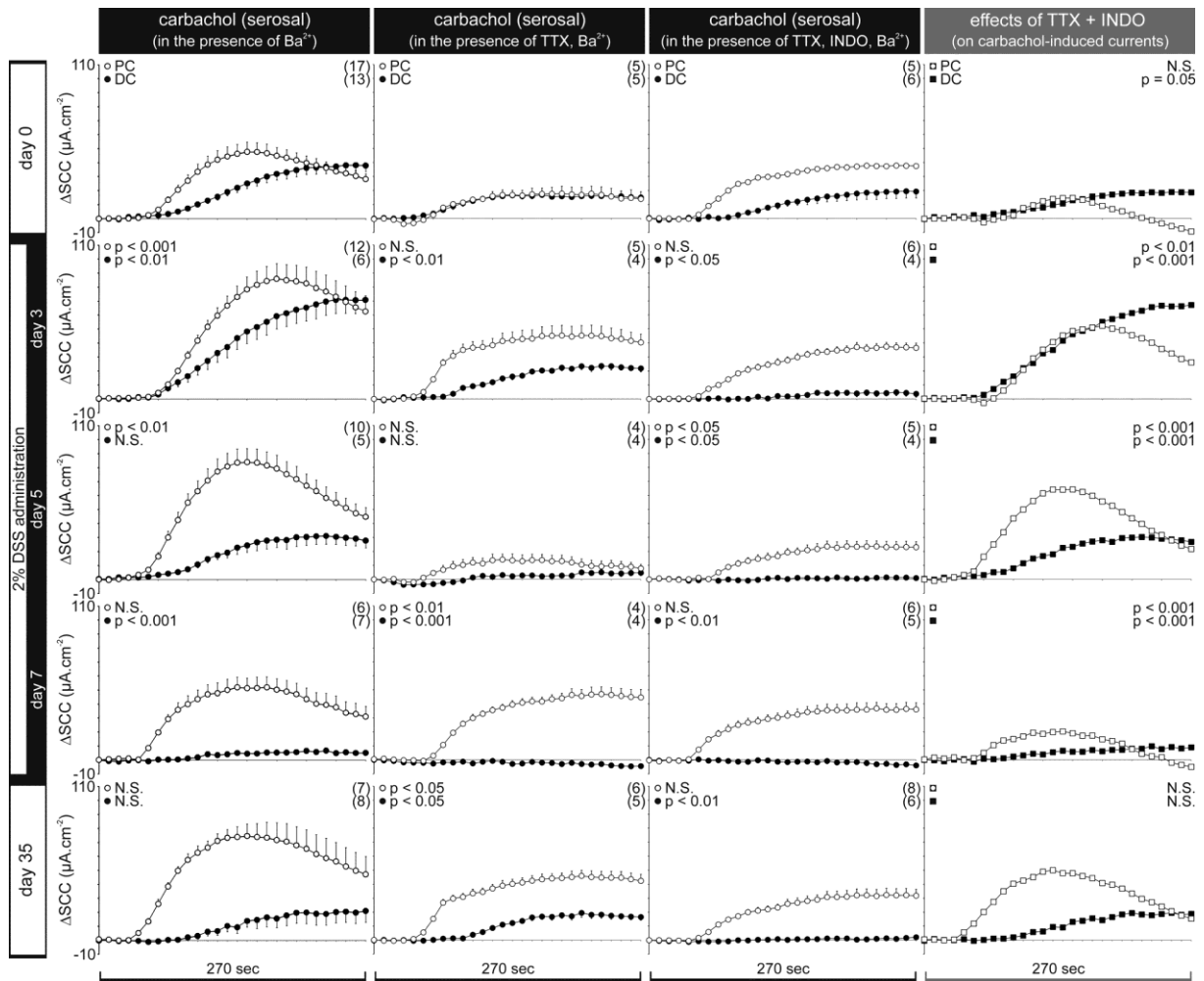


Fig. 5.

