Uterine motor alterations and estrous cycle disturbances associated with colonic inflammation in the rat

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Running head: Uterine contractility and estrous cycle in colitis

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ABSTRACT

The impact of colitis on uterine contractility and estrous cycle was investigated after intracolonic administration of 2,4,6-trinitrobenzenesulfonic acid (TNBS) in rats. Colitis severity was assessed by macroscopic damage scoring (MDS) 4 days after TNBS, and myeloperoxidase (MPO) activity measured in both colon and uterus of control and colitic rats. Estrous cycle stages were determined by vaginal smears and histology, and uterine contractility assessed in vitro on longitudinal and circular strips. In control rats, uterine MPO activity varied markedly during the cycle and peaked around estrus. In rats with moderate colitis (MDS<5, mean±SEM: 3.1±0.2), uterine MPO decreased by 61% compared to estrus control, without disruption of the cycle. Frequency of spontaneous contractions was reduced by 32% in circular muscle. Contractile responses to KCl and carbachol were not affected, while maximal response to oxytocin decreased by 47% in the longitudinal muscle. In rats with severe colitis (MDS>5, 6.0±0.2), uterine MPO was reduced by 96% and estrous cycle disrupted. Spontaneous contractility was impaired in circular strips, and a 39% decrease in the contraction frequency occurred in the longitudinal strips. Circular strips did not contract to KCl or carbachol, while maximal responses to KCl, carbachol and oxytocin of longitudinal strips were reduced by 36%, 27% and 46%, respectively. Estrogen replacement protected the uterine responses to carbachol in colitic rats, whereas oxytocin responses remained depressed. These data indicate that colonic inflammation can influence both spontaneous and evoked uterine contractility, in relation to estrous cycle disturbances, impaired estradiol production and functional alterations of myometrial cells.

Key words: colitis; female genital tract; oxytocin; acetylcholine; viscero-visceral interaction
INTRODUCTION

It is well accepted that hormonal fluctuations across the menstrual cycle influence the gastrointestinal symptoms related to irritable bowel syndrome (IBS) and inflammatory bowel diseases (IBD) (15, 22). Conversely, the impact of bowel pathology on the physiology of the female reproductive organs is poorly understood. Nonetheless, clinical observations have evidenced the occurrence of menstrual abnormalities (including amenorrhea, oligomenorrhea and dysmenorrhea) and/or subfertility in active IBD patients, especially in those suffering from Crohn’s disease (2, 7, 21, 49), suggesting a pathophysiological relationship.

In animal studies, viscero-visceral interactions involving both genital and gastrointestinal tracts have not been reported. However, intestinal inflammatory processes involve the secretion of various inflammatory mediators with pleiotropic biological activities. For instance, a number of these substances are also physiologically produced in genital organs (25, 44). Indeed, animal and human studies show a pivotal role of pro-inflammatory mediators in the control of steroidogenesis and ovulation (5, 12), or in the initiation and the maintenance of uterine contractions at parturition (25, 26). Conversely, studies in the monkey and sheep indicate disturbances in steroidogenesis and ovarian cyclicity following endotoxin administration, considered as a model of systemic inflammation (23, 49, 50). Furthermore, in vitro studies on myometrial cells have investigated the effects of pro-inflammatory mediators on oxytocin (OT) function, a potent uterotonic agent (39). These include an increase or a decrease of OT signaling and receptors, for short- and long-term exposure to inflammatory conditions, respectively (36, 42). However, these studies addressed the OT receptor function on cultured myometrial cells, but the uterine contractile response to OT in inflamed animals remains to be investigated. From all these data, one may hypothesize that a prolonged exposure of the genital tract to an inflamed area in the neighboring colon could affect ovarian and uterine functions.
The aim of the present study was therefore to investigate the effects of trinitrobenzene sulfonic acid (TNBS)-induced colitis on the ovarian cyclicity and uterine contractile activity in the rat. In view of a possible estrous cycle disruption during colitis, the complete achievement of the sexual cycle was assessed through daily vaginal smears and histological control of the uterus. The activity of the enzyme myeloperoxidase (MPO), an estimate of neutrophil infiltration, was measured in both the uterus and colon of control and colitic rats i/ to determine the tissue contents in neutrophils throughout the sexual cycle, and ii/ to highlight the putative modifications in the uterus following colonic inflammation. Both spontaneous and OT-evoked contractions were examined during the estrus period, where the uterus displays regular spontaneous contractions (17, 19), and high responsiveness to OT (28). Because the cholinergic innervation is an important regulatory mechanism of uterine activity (17, 41), the in vitro contractile response to carbachol (Carb), a muscarinic cholinergic agonist, was also studied.

MATERIAL AND METHODS

Animal preparation

Experiments were performed on adult female Sprague-Dawley rats (Charles River, Saint-Aubin-Lès-Elbeuf, France), weighing 225-250 g. Rats were housed in groups of 4 per cage with free access to food and water, under a 12h:12h light:dark cycle, lights on at 8h00 a.m.. In a first group of 40 rats, estrous cycle stages were assessed through daily vaginal smears (13), collected on glass slides and stained with Giemsa (Sigma, Saint-Quentin Fallavier, France). Ten rats in proestrus, estrus, metestrus and diestrus stages were used for determination of basal MPO activity in the uterus and colon during the sexual cycle in healthy circumstances. In a second group of 70 rats in estrus, MPO activity in the uterus and colon (n=20), and uterine contractility (n=50) were investigated after induction of the experimental colitis. A third group of 15 rats was bilaterally ovariectomized (OVX)
under deep anaesthesia with ketamine hydrochloride (150 mg/kg i.p., Imalgène 500, Rhône Mérieux, Lyon, France). After a recovery period of 6 days, long enough to get a complete depletion of endogenous hormones, rats were re-anaesthetised and a Silastic® capsule containing estradiol benzoate (EB) (1,3,5[10]-estratriene-3, 17β-diol-3 benzoate; Sigma) was implanted under the skin of the neck. The EB implant (0.062 i.d., 0.125 o.d.; Dow Corning, Midland, Michigan, USA) was 10mm/100g body weight to obtain physiological plasma levels of estradiol (46). All OVX+EB rats were used for evaluation of uterine contractility following experimental-induced colitis. All protocols were performed in compliance with the European laws on the protection of animals (86/609/EEC), and approved by the local institutional animal care and use committee.

**Inflammation procedure**

In order to induce inflammation in the distal colon, rats were anaesthetised with ketamine hydrochloride as above, and a volume of 0.1 ml of trinitrobenzene sulphonic acid (TNBS) (Sigma; 40 mg.kg⁻¹ diluted in 50% ethanol) was instilled into the lumen of the colon using a polyvinyl rubber catheter (2mm o.d.) inserted rectally 7 cm proximal to the anus (30). Control rats received an equivalent volume of sterile saline since both ethanol and TNBS are inflammatory agents (20). Animals were killed by cervical dislocation 4 days after TNBS or saline treatments. In cyclic rats, all instillations were carried out at the estrus stage so that they were sacrificed 4 days later i.e. on the estrus day of the following cycle, since a complete sexual cycle lasts about 4 days in the rat (13, 19).

**Macroscopic damage scores**

After sacrifice, the scoring of colonic macroscopic damage was done according to the method of Wallace et al (47) slightly modified. Briefly, the presence of mucosal hyperemia and of bowel wall thickening, the severity and extent of ulceration and tissue adhesion, and the occurrence of diarrhea
were rated according to a macroscopic damage score (MDS) ranging from 0 (normal appearance) to 13 (severe lesions) as previously described (30).

Myeloperoxidase activity

The activity of the enzyme MPO, a marker of polymorphonuclear neutrophil primary granules, was determined in colonic and uterine tissues, according to a modified method of Bradley et al (6). Segments of colon and uterine horns (about 0.5 cm each) were suspended in potassium phosphate buffer (KPB, 50 mM, pH 6.0), and homogenized on ice using a Polytron. Three cycles of freezing and thawing were done and suspensions were then centrifuged at 13 000 rpm for 15 min at 4°C. Supernatants were discarded and pellets were resuspended in the detergent hexadecyl trimethylammonium bromide buffer (HTAB 0.5%, p/v, in KPB; Sigma) to release MPO from the primary granules. After sonication on ice, suspensions were centrifuged at 13 000 rpm for 15 min at 4°C, and supernatants assayed spectrophotometrically for MPO activity and protein content. Supernatants were diluted in KPB containing 0.167 mg/ml of O-dianisidine dihydrochloride and 0.0005% of hydrogen peroxide. Absorbance at 450 nm was recorded with Uvikon 860 spectrophotometer (Kontron Instruments, Saint Quentin en Yvelines, France) at 10 s intervals over 2 min. Myeloperoxidase of human neutrophils (0.1 unit/100 µl) was used as a standard. The absorbance change at 450 nm for 1µmol hydrogen peroxide/min at 25°C was calculated from the standard curve, and equals 1 unit of MPO activity. Protein concentrations were determined by the method of Lowry (Bio-Rad Detergent Compatible Protein Assay, Bio-Rad, Ivry, France), and MPO activity expressed as MPO units/g of protein.

Histology
Uterine segments (1 cm length), excised from mid uterine horn in cyclic rats of the control and inflamed groups, were fixed in Bouin’s solution, cleared in xylene and embedded in paraffin. Transverse tissue sections (5 µm) were processed for routine histological analysis with hemalun and eosin staining.

**In vitro contractility**

Uterine strips of 4 mm long were prepared from mid uterine horns of inflamed and control rats. Tissues were mounted in organ bath of Krebs solution (2.8 mM glucose; 6.2 mM KCl; 144 mM NaCl; 2.5 mM CaCl₂; 0.5 mM MgSO₄; 1 mM NaH₂PO₄; 30 mM NaHCO₃) at 30°C, continuously bubbled with 95% O₂+5% CO₂. Depending on the orientation of the uterine strips in the organ bath, we measured isometric contractions of longitudinal muscle (LM) or circular muscle (CM) using a Bioscience UF1 tension transducer (Phymep, Paris, France) under 0.5g resting force. A 45 min equilibration period was allowed before experiments. In a first group of cyclic rats instilled with saline (n=7) or TNBS (n=10), longitudinal and circular uterine strips were exposed to a 40 mM KCl-induced depolarization, considered as producing the maximal contraction of the uterine musculature (33). In all other animals, mechanical responses to cumulative doses of Carb (10⁻⁸M to 10⁻⁴M) (carbamylcholine chloride; Sigma) or OT (10⁻¹⁰M to 10⁻⁵M) (Sigma) applied every 3 min were examined in each tissue preparation. Concentration/response curves were constructed by computerized calculation of the integral under the tension-time curve for 3 min. Isometric changes in tissue tension and maximal effects (Eₘₐₓ) were expressed as a percentage above the spontaneous activity in the absence of drugs, or in g per mg of tissue over resting force, and potency as EC₅₀ (µM).

**Data analysis**
Data were expressed as mean±SEM. Difference in MPO activity in the uterus and colon throughout the estrous cycle was assessed by one way ANOVA followed by Bonferroni post-test. Differences in MPO activity, and in $E_{\text{max}}$ and $E_{C50}$ values from concentration-response curves between two groups were tested using two-tailed Student’s $t$-test for unpaired data. Statistical analysis were performed by running Prism 4 Software (GraphPad, San Diego, CA). A p<0.05 was considered as significant.

RESULTS

*MPO activity in the uterus and colon throughout the estrous cycle*

Myeloperoxidase activity in the uterus varied significantly throughout the cycle (range 2932±575 to 7558±1335 U/g of proteins, n=10 per stage), with highest values measured in proestrus and estrus stages (Figure 1A). No significant change was observed from proestrus to estrus, while MPO activity decreased significantly in metestrus and diestrus (p<0.01 and p<0.05 compared with proestrus, respectively). In the colon from the same cyclic rats, the basal MPO activity did not significantly vary from proestrus to diestrus stage (Figure 1B).

*Effect of TNBS-induced colitis on estrous cycle*

*Macroscopic colonic damages and estrous cycle stages.* Four days after intrarectal administration of saline to estrus rats, no alteration of the colonic mucosa was noted (n=28). Rats instilled with saline were in estrus stage of the following sexual cycle, as revealed by the only presence of large keratinized cells on vaginal smears (not shown). In contrast, 52.4% (n=22) of rats instilled with TNBS did not display estrus stage 4 days after treatment, but a persistent luteal phase. In these animals, the macroscopic colonic damage consisted of several inflammation sites with ulcerations in an area extended from 1 to 3 cm around the instillation point. Necrotic areas were often observed
throughout the inflamed colonic region, with liquid feces in the colon, corresponding to a macroscopic damage score (MDS) of 6.0±0.2 (range 5 to 9). The remaining animals instilled with TNBS (n=20) displayed estrus stages on vaginal smears, and showed local hyperemia in the colon and/or bowel wall thickening without ulcers or diarrhea, corresponding to a MDS of 3.1±0.2 (range 1 to 4).

_Uterine histology._ The uterine horn of control estrus rats exhibited a thick muscular wall, composed of an outer longitudinal and inner circular myometrial layer, and vascular bed between the two layers (Figure 2A). The endometrium revealed numerous endometrial glands, a thick epithelium composed of columnar cells exhibiting elongated nuclei, while the uterine lumen displayed complex and deep invaginations within the endometrial stroma. No morphological changes were observed in rats instilled with TNBS and displaying a MDS<5 in the colon (Figure 2B). In contrast, animals with a MDS>5 showed a decrease of the diameter of the uterine horn, reduced endometrial stroma and hypotrophy of the epithelium with general loss of invaginations (Figure 2C).

_Colonic and uterine MPO activities._ In basal conditions, the MPO activity in the colon and uterus from estrus rats instilled with saline was of 199±50 and 7240±1310 U/g of protein, respectively. TNBS instillation increased colonic MPO activity in all treated rats (Figure 3A), and this increase was significantly higher (p<0.05) in rats with a MDS>5 when compared with animals displaying a MDS<5 (2990±613 vs 914±52 U/g of protein). Conversely, the MPO activity was significantly decreased in the uterus from the same inflamed rats (Figure 3B), by 61% in rats with a MDS<5 (2800±336 U/g of protein; p<0.05 compared to control), and nearly suppressed (-96%) when a MDS>5 was scored in the colon (258±70 U/g of protein; p<0.01 compared to control).

_Effects of TNBS-induced colitis on uterine contractility_
Spontaneous contractions. Four days after colonic instillation with saline, all uterine strips mounted in the organ bath exhibited rhythmic spontaneous contractions. The frequency, amplitude and duration of the contractions varied, depending on whether recording was obtained from the longitudinal or circular muscle layer (Figure 4). The most distinctive difference occurred in the contraction frequency, the highest occurring in the circular musculature (Table 1). In the TNBS treated group, rats characterized by moderate colonic damages (MDS<5) exhibited a significant decrease in the frequency of uterine contractions (-32%) in the circular muscle, while no change occurred in the longitudinal layer (Figure 4A2, B2 and Table 1). A significant decrease (-39%) in the frequency of contractions of the longitudinal muscle was observed only in rats with severe colonic injury (MDS>5) (Figure 4 A3 and Table 1). In the same animals, the effects were more pronounced in the circular musculature, showing irregular tracing of spontaneous activity without rhythmic contractions (Figure 4 B3).

Uterine response to KCl. Application of KCl induced non-receptor mediated contractions of the uterine strips, caused by a direct depolarization of the smooth muscle cells. In control animals, maximal contraction was obtained after 40 mM KCl application. Four days after intracolonic instillation of TNBS, in rats showing moderate colonic damage (MDS<5), no differences were noticed in the contractile response to 40 mM KCl compared with controls in either longitudinal or circular uterine strips (Figure 5). In contrast, when severe colonic damage occurred (MDS>5), the response to KCl was significantly decreased (-36%) in longitudinal strips (156±33 vs 244±8 % in control; p<0.01), while circular muscle preparations from the same TNBS-treated animals were unresponsive to KCl (Figure 5).

Uterine response to carbachol. Carb caused a concentration-dependent increase of contractile activity in both longitudinal and circular muscle strips from control rats (Figure 6), with a mean
EC\textsubscript{50} of 3.7±0.4 µM in the longitudinal and 2.4±0.5 µM in the circular strips, and E\textsubscript{max} obtained at 100 µM in both muscle layers. TNBS-treated rats with a MDS<5 in the colon showed no significant difference in the contractile response to Carb in either longitudinal or circular strips when compared with controls (Figure 6A, B). In contrast, in rats with severe colonic damages (MDS>5), the E\textsubscript{max} in longitudinal uterine strips was significantly decreased by 27% when compared to control value (163±12 vs 222±16 %, p<0.05), without affecting the EC\textsubscript{50} (5.5±0.6 M, p>0.05) (Figure 6A). Circular muscle preparations from the same animals did not respond to Carb (Figure 6B).

\textit{Uterine response to oxytocin.} OT significantly increased contractions of longitudinal strips, while the circular preparations were less sensitive to OT stimulation (not shown). This is in agreement with the predominant localization of OT receptors in the longitudinal layer of myometrium (31). Thus, all subsequent experiments with OT were conducted in the longitudinal musculature. In control rats, the E\textsubscript{max} induced by OT was 269±20 % and obtained at 100 nM OT. In TNBS-treated rats, the maximal response to OT was markedly decreased (Figure 6C), in a similar extent in animals with either moderate or severe colonic lesions (-47% and -46%, respectively; p<0.01 compared with control). In contrast, there was no difference in EC\textsubscript{50} values between control (2.1±0.5 nM) and TNBS-treated rats (1.6±0.6 and 3.6±0.8 nM for low and high MDS, respectively).

\textit{Influence of estradiol on uterine responses to Carb and OT}

In OVX+EB rats, the uterine contractile response to Carb was assessed primarily in the circular muscle preparations, where changes in the muscarinic receptor stimulation were found the most pronounced in cyclic rats treated with TNBS (see above). After equilibration, the circular strips in OVX+EB rats was characterized by an almost flat tracing of spontaneous activity or exhibited scarce and erratic contractions (not shown). Thus all subsequent analysis of isometric tension
changes were expressed as increases over the basal tension, and normalized per tissue weight. In OVX+EB rats instilled with TNBS (Figure 7A), the maximal response to Carb was not affected whatever the extent of colonic lesions (0.20±0.01 and 0.23±0.04 g/mg of tissue in MDS<5 and MDS>5 groups, respectively) when compared with saline treated OVX+EB rats (0.23±0.01 g/mg of tissue, p>0.05), and did not differ from that observed in estrus animals treated with saline (0.19±0.02 g/mg tissue, p>0.05). In contrast, when the OT stimulation was tested in the same TNBS-treated animals (Figure 7B), the EB treatment did not restore the maximal response in the longitudinal muscle layer (0.22±0.02 and 0.21±0.04 g/mg of tissue in MDS<5 and MDS>5 groups, respectively), about 45% lower than that observed in saline OVX+EB (0.38±0.04 g/mg of tissue, p<0.05) or estrus rats (0.36±0.03 g/mg of tissue, p<0.05).

**DISCUSSION**

The present study provides first evidence in rats that colitis induces uterine motor alterations and estrous cycle disturbances. We also report that 4 days after colonic instillation of a single dose of TNBS (40 mg/kg) –a time course permitting the complete achievement of a sexual cycle in the rat (19)- disruption of the cycle was only observed in rats showing severe tissue injuries in the colon. In considering that macroscopic damage in the colon vary between individuals from an inflamed area localized at the injection site to a necrosis affecting large region in the distal colon, our study has investigated pathophysiological changes in the reproductive tract in relation to the severity of colonic inflammation.

Data concerning the increase of MPO activity in the colon after TNBS treatment reflect the mucosal infiltration of polymorphonuclear neutrophils in response to inflammation (30). The fact that colonic MPO activity peaked in rats with severe colitis emphasizes that the degree in neutrophil infiltration correlates well the magnitude of the inflammatory response. In comparison, the MPO activity measured in the uterus of colitic rats clearly demonstrated that no inflammatory response
was initiated in the genital tract during the course of colitis, whatever the extent of tissue damage in
the colon. This result eliminates the hypothesis of a systemic diffusion of TNBS, which could affect
the neighboring organs in the pelvic/abdominal cavity. Conversely, we show that colitis induced a
dramatic decrease in uterine MPO when compared with control estrus rats, to reach its nadir in
animals with severe colitis. In healthy individuals, it is of interest to note that uterine tissues
exhibited high basal levels of MPO activity, reaching their maximum during the follicular phase of
the cycle, i.e. at the proestrus and estrus stages. The elevated basal MPO in the uterus agrees with
reports in rats and humans demonstrating that the uterine tissue remodeling throughout the cycle
resembles an inflammatory response, with transient neutrophil infiltration (24, 40). Our observation
that basal uterine MPO peaked during the follicular phase is consistent with the massive influx of
neutrophils in this organ near estrus, due to a pro-inflammatory effect of estrogen (24, 45), the
predominant hormone at this stage of the estrous cycle. In comparison, the present study also
indicates that cyclic changes in ovarian steroids did not interfere with the basal MPO activity in the
colon, demonstrating that colonic neutrophil populations in healthy conditions are insensitive to the
steroid environment. The finding that colitis impairs the normal rise of uterine MPO in estrus
indicates that the gut inflammatory response is able to block the cycle-dependent changes in uterine
neutrophil populations. Moreover, we found that colitic rats exhibited a persistent luteal phase on
vaginal smears in the case of severe inflammation, while histological findings on uteri revealed that
the normal developmental sequence leading to an increase in the thickness of endometrium and
luminal epithelium was impaired when compared to healthy estrus rats. Taken together these
observations demonstrate that severe colonic inflammation disrupts the ovarian cyclicity and
associated uterine tissue remodeling. The mechanisms underlying this viscer-visceral interaction
likely involve influence of inflammatory mediators on the ovarian steroidogenesis. Indeed, colon
inflammation is associated with an increase in circulating cytokines, mainly interleukin (IL)-1β, IL-
6, IL-8, and tumor necrosis factor alpha (TNF-α) (14), although plasma levels of TNF-α remain low in the rat model of TNBS-induced colitis (32). Studies in rats have shown that the IL-1β - which is produced in large amounts during acute colitis in rats and humans (27, 34) - prevents the preovulatory increase in estradiol secretion, and has cytotoxic effects on cultured ovarian cells (12, 18, 38). Similar mechanisms for estrous cycle disruption have been reported in the ewe following endotoxin administration (23). In the current study, although estradiol levels were not measured, impairment of its secretion is consistent with the inhibition of neutrophil accumulation and uterine tissue growth.

The \textit{in vitro} uterine spontaneous contractility showed alterations that paralleled the severity of colitis, with marked differences between muscle layers. These alterations were characterized by a decreased contraction rate in the circular muscle during moderate inflammation, without changes in its longitudinal counterpart. In the longitudinal musculature, a similar alteration became evident only when the estrous cycle was impaired following severe colitis, while the circular muscle was unable to produce reproducible rhythmic contractions in the same animals. It is likely that these effects were closely related to ovarian cycle disturbances, as pointed above. Indeed, the spontaneous activity consists of myogenic contractions, with variable amplitude, duration and frequency throughout the estrous cycle in relation to hormonal changes (19). From \textit{in vitro} studies, we previously reported a higher contractile rate in uterine strips during estrus as compared to diestrus stage (17). In the same study, we also showed that estrogen dominance mainly enhanced the contraction rate in the circular layer as compared to the longitudinal one, which could explain why the circular muscle appeared more affected in colitic rats in the present report. Furthermore, the frequency of longitudinal muscle contractions during severe colitis was lowered to a level normally observed in diestrus stage of cyclic healthy rats (0.61±0.03 contraction.min$^{-1}$ in the present study \textit{versus} 0.60±0.02 in diestrus (17)), with similar durations. This emphasizes that the motor pattern in
this uterine musculature correlates well the persistent luteal phase reported here when severe colitis occurred. Finally, the circular musculature was found unresponsive to KCl depolarization, whereas the longitudinal muscle was still able to contract, however to a lesser extent than observed in control estrus rats. The decreasing response to KCl depolarization was not unexpected, since estrogen plays a key role in promoting calcium uptake by myometrial cells (3), i.e. the second messenger of uterine contractility (44), that largely contributes for potassium depolarization-mediated contractions (4).

Furthermore, it has been shown that induction of myometrial gap junctions, permitting the cell-to-cell propagation of contractions (44), required high levels of estrogen (9), mainly in the circular muscle cells where gap junctions were found more concentrated than in its longitudinal counterpart (9, 37).

When the myometrial response to Carb was examined, only rats with severe colitis revealed a defect in the contractile response to the muscarinic agonist. In line with KCl responses, the circular muscle was found unresponsive to Carb, while the longitudinal layer was less affected, so that similar mechanisms for impaired KCl stimulation may also explain the altered Carb responses. Indeed, we have recently shown in cyclic rats that estrogen dominance at the estrus stage enhances myometrial sensitivity to muscarinic stimulation, while Carb was found ineffective to induce contraction in diestrus, i.e. under progesterone dominance (17). Furthermore, estrogen enhances intracellular signalling pathways linked to activation of muscarinic receptors in rat myometrial cells (1), and we report herein that estradiol replacement in ovariectomized rats totally protected the muscarinic stimulation in animals subject to severe colitis. Accordingly, the uterine muscarinic desensitization in our study appeared as a consequence of decreased levels of endogenous estradiol. In contrast, a decreased OT stimulation was observed in all TNBS-treated rats, i.e. whatever the extent of colon damage, indicating that these effects did not depend on the severity of colitis as for KCl and Carb. Moreover, estrogen treatment was found ineffective in restoring the uterine responses to OT, suggesting a distinct inhibition of this uterotonic pathway. For instance, decreased OT
receptor density and impaired OT intracellular signaling have been recently demonstrated in cultured myometrial cells following a prolonged exposure to IL-1β and IL-6 (16, 35, 36, 42), even though controversial data exist regarding the deleterious effect of IL-6 on OT receptor expression (16, 42). Although OT receptor densities were not measured in our study, myometrial OT desensitization during colitis may involve similar processes, where increased levels of plasma IL-1β and IL-6 during colitis could result in an inhibition of the OT response of the uterus.

In summary, these data provides experimental evidences for pathophysiological relationships between digestive and reproductive tracts during colitis in the cyclic rat. This viscero-visceral interaction is able to disrupt the sexual cycle, as evidenced by the cascade of perturbations in endometrial and epithelial growth, neutrophils accumulation and myometrial activity, both the spontaneous and evoked contractions. Based upon the protective effects of exogenous estrogen reported herein, and because sex steroids regulate uterine growth and myometrial activity, it is likely that most of the perturbations elicited by colitis result from a defect in ovarian estradiol production, while alterations in uterine response to OT suggest a direct systemic action of pro-inflammatory mediators on OT receptor expression. Further studies are required to test this hypothesis. During the sexual cycle, myometrial contractions at the time of conception are essential for sperm transport towards the site of fertilization, and later on, for blastocyst implantation (8, 11, 29). Moreover, the uterine tissue remodeling throughout the cycle is a key step in the preparation to gestation (10). Thus, our findings in a rat model of colitis may have correlates in human IBD, for unexplained associations with ovarian dysfunction and reduced fertility, occurring mostly during active state of diseases (7, 48).
REFERENCES


FIGURE LEGENDS

Figure 1: Variation of myeloperoxidase (MPO) activity in the uterus (A) and colon (B) from proestrus to diestrus stages of the sexual cycle in healthy rats. *p<0.05, **p<0.01, ns: not significant versus proestrus stage (values are means±SEM, n=10).

Figure 2: Cross sections from the mid uterine horn at low (A1-C1) and high (A2-C2) magnification obtained from control oestrus rats treated with saline (A) and rats 4 days after intracolonic administration of TNBS and showing (B) moderate (MDS<5) or (C) severe colon damage (MDS>5). The development of endometrium (En) and the thickness of myometrium (Myo) were reduced during severe colitis only (C) as compared to control uterine horn (A). Note the decrease in diameter of the uterine horn (C1) and general hypotrophy of epithelial cells (Ep) (arrows in C2) in rats displaying severe colon damage. Micrographs in each column are at the same final magnification. MDS: macroscopic damage score in the colon. UL: uterine lumen; EG: endometrial glands.

Figure 3: Effect of TNBS-induced colitis on myeloperoxidase (MPO) activity in the colon (A) and uterus (B) of rats showing moderate (MDS<5) or severe colon damage (MDS>5). MDS: macroscopic damage score in the colon. *p<0.05 and **p<0.01, significantly different from the corresponding saline control. Differences between groups: a, p<0.05 and b, p<0.001 (values are means±SEM ; n, number of rats).

Figure 4: Representative recordings of spontaneous contractions in the longitudinal (LM) (A) and circular (CM) (B) muscle layers of rat uterus 4 days after intracolonic instillation of saline (A1, B1) or TNBS and showing moderate (A2, B2) or severe colon damage (A3, B3). MDS: macroscopic
damage score in the colon.

Figure 5: Maximal contractile response to KCl (40 mM) of the longitudinal (LM) and circular (CM) muscle layers of rat uterus 4 days after intracolonic administration of saline or TNBS. Data are expressed as percentage above spontaneous activity (± SEM), and points are means of 4-8 replicates per animal in 5-7 rats per group. **\( p < 0.01 \) and ***\( p < 0.001 \) versus corresponding saline control. MDS: macroscopic damage score in the colon.

Figure 6: Contractile activity of the circular (CM) and longitudinal (LM) muscle in response to increasing concentrations of carbachol (Carb) (A, B) and oxytocin (OT) (C) 4 days after intracolonic instillation of saline or TNBS. MDS: macroscopic damage score in the colon. Data are expressed as percentage above spontaneous activity (± SEM), and points are means of 4-8 replicates per animal in 6-8 rats per group. *\( p < 0.05 \) and **\( p < 0.01 \) versus saline-treated control rats.

Figure 7: Maximal contractile response of the circular muscle (CM) to carbachol (A) and of the longitudinal muscle (LM) to oxytocin (B) 4 days following intracolonic instillation of saline or TNBS in oestrus rats and in oestradiol benzoate-treated ovariectomized rats (OVX+EB). Data are expressed as g of tension over resting force per mg of tissue weight (± SEM), and points are means of 4-8 replicates per animal in 4-6 rats per group. *\( p < 0.05 \), **\( p < 0.01 \), and ***\( p < 0.001 \) versus corresponding saline control. MDS: macroscopic damage score in the colon.
Table 1. *Frequency of spontaneous contractions in uterine muscle strips after intracolonic instillation of saline or TNBS*

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<th>Saline</th>
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<td><strong>Contraction frequency (min⁻¹)</strong></td>
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<td>LM</td>
<td>1.00±0.08</td>
<td>0.95±0.09&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>0.61±0.03&lt;sup&gt;‡&lt;/sup&gt;</td>
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<td>n=14</td>
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<td>CM</td>
<td>1.47±0.11&lt;sup&gt;*&lt;/sup&gt;</td>
<td>1.00±0.18&lt;sup&gt;†&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>n=16</td>
<td>n=10</td>
<td>n=10</td>
</tr>
</tbody>
</table>

Values are mean±SEM from 3-4 replicates per rat. MDS: macroscopic damage score in the colon; LM: longitudinal muscle; CM: circular muscle; n: number of rats; * p<0.01 versus longitudinal muscle; † p<0.05, ‡ p<0.001 compared to respective saline control; ns: not significant. ND: not determinable.
**FIGURES**

**Figure 1**

**A**

![Graph A showing MPO (units/g protein) levels for proestrus, oestrus, metestrus, and diestrus stages with ns, **, and * symbols indicating significance differences.]

**B**

![Graph B showing MPO (units/g protein) levels for proestrus, oestrus, metestrus, and diestrus stages with error bars indicating variability.]

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*ns, **, * symbols indicate significance differences.*
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6

A  LM

B  CM

C  LM

Uterine contraction (%) vs Log[Carb] (M)

-5 -4 -3 -2 -1 0 1 2 3 4 5

Saline  MDS<5  MDS>5  TNBS

-5 -4 -3 -2 -1 0 1 2 3 4 5

Saline  MDS<5  MDS>5  TNBS

-11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0 1 2 3 4 5

Saline  MDS<5  MDS>5  TNBS
Figure 7

A
CM
Maximal response to carbachol

B
LM
Maximal response to oxytocin