Estrogen altered visceromotor reflex and P2X₃ mRNA expression in a rat model of colitis

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P2X₃ and P2X₂/₃ receptors are expressed in peripheral tissues and dorsal root ganglia (DRG) and participate in peripheral pain. However, the mechanisms underlying P2X receptor-mediated nociception at different ovarian hormone levels have not been examined. In this study, 24 female rats were randomly divided into sham-operated (sham), ovariectomized (OVX), estrogen-treated, and estrogen–progesterone-treated groups with colitis. In each group, the visceromotor reflex (VMR) to colorectal distension was tested and the DRG were harvested for a real-time PCR analysis of P2X₃ and P2X₂ receptor mRNA. In OVX rats with colitis we found that the VMR to colorectal distension and P2X₃ receptor mRNA in DRG were both significantly decreased. Estrogen replacement reversed the decrease. However, neither the VMR nor the P2X₃ mRNA level in DRG from OVX colitis rats was reversed by the complex of estrogen and progesterone. Patch-clamp recording showed that in colitis rats, estradiol rapidly potentiated the sustained and transient currents evoked by ATP to 336 ± 49% and 122 ± 12% of controls, respectively, in a subpopulation of DRG neurons, which were blocked by ICI 182, 780, an antagonist of the estrogen receptor. Whereas progesterone rapidly inhibited the transient currents induced by ATP to 67 ± 10% of control and had no effect on the sustained currents evoked by the same agonist. These results indicate that P2X₃ receptors are likely to be an important contributor to the altered colonic functions in colitis rats, where the underlying mechanisms are closely related to endogenous estrogen modulation.

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1. Introduction

About 4,000,000 people in the world suffer from inflammatory bowel disease (IBD) [1]. IBD is a group of inflammatory conditions of the colon and small intestine. The major types of IBD are Crohn’s disease and ulcerative colitis. Though persons with IBD and/or irritable bowel syndrome (IBS) share similar signs and symptoms (abdominal pain, alterations in form, and frequency of stool) [2], visceral hypersensitivity plays an important role in IBS and may account for symptoms of urgency, bloating, and abdominal pain [3–6]. In the current study, we used colonic trinitrobenzene sulfonic acid (TNBS) inflammation as a trigger for visceral hypersensitivity associated with ovariectomy and female hormones replacement.

It has been reported that a wide variety of signaling molecules are involved in initiating and maintaining the inflammatory response, including cations, amines, kinins, prostanoids, purines, cytokines and growth factors. Adenosine 5′-triphosphate (ATP) is an important candidate which plays a role in nociception and, in particular, inflammatory pain. Behavioral studies in rats [7,8] and humans [9] have demonstrated that the pain-inducing effects of ATP are enhanced in states of inflammation. Nerve recordings also show exaggerated responses to ATP from inflammatory tissues [10]. ATP acts via both ionotropic P2X receptors and metabotropic P2Y receptors. P2X receptors are expressed in peripheral sensory nerve terminals and in small-diameter sensory neurons in dorsal root ganglia (DRG), which are known to supply, among other areas, the pelvic viscera [11–13]. P2X₃ receptor-null mice show reduced formalin-induced pain behavior [14,15] and P2X₃ receptors are upregulated in colitis specimens obtained from patients with IBD [16] and in animal models of colitis [39] and IBS [17]. ATP released during distension from epithelial cells lining tubes (such as ureter or gut) and sacs (such as bladder) acts on P2X₃ and/or P2X₂/₃ receptors on a subepithelial nerve plexus to initiate impulses that are relayed via the spinal cord to pain centers in the brain [13,18].

It is also reported that the chronic pain conditions, such as IBS, are more prevalent in females than in males and the severity of pain fluctuates with the menstrual cycle [19,20], suggesting that gonadal hormones may affect the perception of painful stimulations. However, clinical data on the association between IBD and female sex hormones are conflicting. On one hand, IBD is believed...
to flare during a first pregnancy and postpartum and, on the other hand, to be better controlled during subsequent pregnancies [21–23]. Another report suggests that female gender is a risk factor for relapse in ulcerative colitis [24]. Although study design and inclusion criteria may explain some of the discrepancies between studies, a causal relationship between sex hormones and IBD has not been clearly established.

Classical estrogen receptors, which consist of two subtypes (i.e., ER\(_\alpha\) and ER\(_\beta\)) have been described in vascular endothelium, fibroblasts, smooth muscle cells and gastrointestinal mucosal epithelial cells [25–28]. Most of the effects of estrogen are mediated by binding of the hormone to these estrogen receptors (ERs) that act as nuclear transcription activators [26]. T cells, B cells and macrophages are known targets of estrogen [29,30]. Besides, ERs are also widely distributed throughout the central and peripheral nervous systems [31,32]. The peripheral sensory neurons express both estrogen receptor-α (ER\(_\alpha\)) and estrogen receptor-β (ER\(_\beta\)) [33], with ER\(_\alpha\) being selectively localized in small-diameter neurons. Thus it is reasonable to expect that estrogen modulates inflammation at the sensory ganglion level and also in the gastrointestinal system [34].

It has been reported that in ovariectomized rats P2X\(_3\) receptor mRNA levels are significantly increased in rat bladders [35,36]. Our previous studies have also shown that estradiol selectively modulates homomeric P2X\(_3\) receptor-mediated transient currents without affecting homomeric P2X\(_2\) or heteromeric P2X\(_2/3\) receptor-mediated responses in rat cultured DRG neurons [37]. These results suggest estradiol may affect peripheral pain signal transduction at the sensory ganglion level via P2X\(_3\) receptors.

In this study, we examined changes to the visceromotor reflex (VMR) and P2X\(_3\) and P2X\(_2\) receptor mRNA levels in DRG from rats with or without ovariectomy after colitis, and in the female hormone-treated ovariectomized rats, to observe the possible modulatory effects by estrogen during colon inflammation.

### 2. Experimental procedures

#### 2.1. Animals

In VMR and polymerase chain reaction (PCR) experiments, 24 female Sprague–Dawley rats (200–250 g) were randomly divided into four groups. Group 1 included six rats that had a sham operation, group 2 had six rats that had bilateral ovariectomy, group 3 had six rats that had bilateral ovariectomy following 17β-estradiol injection (daily, 30 μg/kg) 1 week after operation and groups 4 had six ovariectomized rats following daily complex (17β-estradiol, 30 μg/kg, progesterone, 50 μg/kg) injection 1 week after operation. After 6 weeks, the rats in each group were used for induction of colitis. Another 16 control and colitis (eight in each group) female rats were used in patch clamp recording. Animals were allowed free access to food and water.

#### 2.2. Induction of colitis

Experimental colitis was induced by administration of an intrarectal enema (8 cm from the anus) of 30% TNBS in ethanol at a dose of 80 mg/kg body weight [38]. The enemas were given through a 6-Fr medical-grade polyurethane enteral feeding tubes while the rats were under light ether anesthesia. This model of chronic inflammation was favored, because this most closely mimics human IBD. Previous work has suggested that, in the TNBS model of colitis in rats, chronic inflammation is evident at day 2 and evolves over several weeks, with the most severe period of inflammation starting at day 5 [38,39]. After 5–7 days of induction of colitis or saline treatment, these rats were subjected to colorectal distension and VMR was tested. After the above experiments, all the rats were killed and the DRG were harvested and analyzed for P2X\(_3\) and P2X\(_2\) subunit mRNA expression. Assessment of colitis was based on body weight, as well as macroscopic and microscopic features of the colorectum.

#### 2.3. Recording of VMR

All rats were implanted with electromyogram (EMG) electrodes 5–7 days before testing. In ether-anesthetized rats, an electrode made from Teflon-coated 32 g stainless steel wire (Cooner Wire Co., Chatsworth, CA, USA) was placed in the lateral abdominal wall, the electrode leads tunneled s.c. and exteriorized at the back of the neck. On the day of experiment, a double-lumen catheter with an 8-cm latex balloon at one port and the other open into the colon was connected to the recorder for pressure measurements from both the balloon and the colon. The balloon was lubricated and placed into the rat’s distal colon so that the tip of the balloon was 2 cm proximal to the anus. A 10 min settling period was allowed in order to achieve a steady level and the rats were not restrained. The intracolonic balloon was manually inflated using an air-filled syringe which allowed slow, continuous inflation. The visceromotor response was measured using 20, 40, 60, 80 mmHg CRD (four distensions at each pressure, 20 s each, 3 min interstimulus interval) [40]. EMG electrodes were connected bilaterally to abdominal muscles to obtain a raw signal of the VMR. The intracolonic and balloon pressures and the EMG trace were continuously recorded after instrument calibration. The visceromotor response (the EMG in response to CRD) was recorded with a CED 1401plus and analyzed using Spike 2 for Windows software (Cambridge Electronic Design, UK). The EMG was rectified offline and the area under the curve (AUC) determined. The baseline visceromotor response was defined as the AUC 10 s prior to distension.

#### 2.4. Measurement of colonic compliance

In awake animals, the volume in the distension balloon was measured by increasing the pressure in the distension balloon by 20 mmHg increments between 0 and 80 mmHg. This was measured three times in each rat, and the mean volume at each pressure increment was used as the measure for that rat.

#### 2.5. The quantitative real-time reverse transcription–polymerase chain reaction analysis

##### 2.5.1. RNA isolation and complementary DNA synthesis

Total RNA from DRG was extracted using a RNeasy Mini Kit (QIAGEN, Clifton Hill, Australia). RNA purity was determined using a method of ultraviolet spectrophotometry at a wavelength of 260–280 nm. Two micrograms of total RNA was reversely transcribed to complementary DNA in a 20 μl reaction mixture containing 1 × reverse transcriptase buffer (15 mM MgCl\(_2\), 375 mM KCl, 50 mM DTT, 250 mM Tris–HCl, pH 8.3), 10 mM dNTP, 20 U RNase inhibitor, 200 U M–MLV reverse transcriptase, and 50 ng of oligo(deoxythymidine)\(_{15}\) primer. Reaction time was at least 1 h at 42 °C. The cDNA was stored at −20 °C until real-time PCR (RT-PCR).

All reagents, with the exception of the RNeasy Mini Kit, were from Promega Corp. (Madison, WI).

##### 2.5.2. Quantitative RT-PCR

Quantitative RT-PCR amplification was performed with SYBR-Green (Applied Biosystems; Scoresby, Australia) using Roto-gene RG3000 (Australia) in 20 μl reaction mixture. The solution consisted of 1.0 μl diluted RT-PCR product, 0.25 μM of each of the paired primers, and 10 μl real-time PCR SYBR Green Master Mix (QIAGEN, Clifton Hill, Australia). RNA levels were measured with
specific primers designed, for P2X3 was: TGGCGTCTGGTATTAAGATCGG (forward); CAGTGGCCTGGTCACTGGCAG (reverse) against the sequences downloaded from GenBank (accession nos. X90651, 708–731 and 1126–1147). The PCR condition was 95 °C for 2 min, followed by 40 cycles of 95 °C, 20 s; 65 °C, 25 s; 72 °C, 25 s. RT-PCR for the housekeeping gene β-actin was performed for each sample. For P2X2 was: GAATCAAGTGGCAACCCAAA (forward); TCACAGGCCATCTACTTGG (reverse) against the sequences downloaded from GenBank (accession nos. U14414, 826–845 and 1183–1164): 95 °C, 2 min; 95 °C, 25 s; 58 °C, 25 s; 72 °C, 25 s. The primer for amplification of β-actin was: ATGTGCTGAGTGCAACCCATA (forward); TGCTGCTGCTGCTGCTGCTGCTG (reverse). The PCR condition was: 95 °C for 2 min, followed by 40 cycles of 95 °C, 20 s; 58 °C, 25 s; 72 °C, 25 s. The absolute mRNA level of target gene in each sample was calculated using a standard curve and then by the ratio to β-actin in each sample. The specificity of the primers was verified by examining the melting curve as well as sequencing of the QT-RT-PCR products. The length of PCR products of P2X3, P2X2 and β-actin mRNA were 440, 357 and 265 bp, respectively.

2.6. Whole-cell patch-clamp recording

Whole-cell currents were recorded at room temperature using an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) with membrane potential being held at −60 mV. External solution contained (in mM): NaCl 154, KCl 4.7, MgCl2 1.2, CaCl2 2.5, Hepes 10 and glucose 5.6 with pH adjusted to 7.4 using NaOH. Recording electrodes (resistance 2–4 MΩ) were filled with internal solution which contained (in mM): citric acid 56, MgCl2 3, CsCl 10, NaCl 10, Hepes 40, EGTA 1, tetraethylammonium chloride 10, and the pH was adjusted to 7.2 using CsOH (total Cs+ concentration 170 mM). Current signals were acquired using pClamp software (version 6.1, Axon Instruments) and were plotted using Origin7 (Microcal, Northampton, MA, USA).

2.7. Chemicals

ATP, 17β-estradiol, progesterone were from Sigma Chemical Co. (Poole, UK), ICI 182, 780 from Tocris Bioscience (Ellisville, MO, USA). Solutions of ATP, 17β-estradiol and progesterone were prepared in deionized water and stored frozen, while ICI 182, 780 was dissolved in dimethylsulfoxide to 1 mM. All drugs were then diluted in extracellular bathing solution to the final concentration.

2.8. Data analysis

The EMG recordings were collected with a CED (Cambridge Electronic Design, Cambridge, UK) and analyzed using Spike 2 for Windows software. The EMG was rectified, and the area under the curve for the 20 s before distension was subtracted from the AUC during the 20 s distension.

Results were represented as mean ± S.E.M. Data in the magnitude of the VMR and compliance were analyzed using two-way ANOVA. One-way ANOVA analysis in SPSS11 was performed in P2X3 and P2X2 receptor expression levels from groups. The multiple comparisons were then made by using the least significant difference (LSD, t-test). Differences among the groups were considered statistically significant when the p value was lower than 0.05 (p < 0.05).

In the patch-clamp recording, statistical analysis (Student’s t-test) was performed using Excel (Microsoft, USA). Traces were acquired using Clampfit (pClamp software) and plotted using Origin7 (Microcal, Northampton, MA, USA).
Fig. 3. (A) The rapid inhibition by 17β-estradiol (1 μM) of ATP (100 μM)-induced transient currents in DRG neurons from control female rats. (B) Diagram showing the effect of 17β-estradiol on ATP-induced transient and sustained inward currents in DRG neurons from control female rats. The rapid potentiation by 17β-estradiol (1 μM) of ATP (100 μM)-induced transient (C) and sustained (D) currents in a subpopulation of DRG neurons from colitis rats. An inhibitor of ER, ICI 182, 780 (1 μM) blocked the rapid effect of 17β-estradiol on transient (C) and sustained (D) currents evoked by ATP (100 μM). (E) Diagram showing the effect of 17β-estradiol on ATP-induced transient and sustained inward currents in DRG neurons from colitis rats and its reversal by ICI 182, 780. *p < 0.05.

Fig. 4. (A) The rapid inhibition by progesterone (1 μM) of ATP (100 μM)-induced transient currents in DRG neurons from control female rats. (B) Diagram showing the effect of progesterone on ATP-induced transient and sustained inward currents in DRG neurons from control female rats. The rapid inhibition by progesterone (1 μM) of ATP (30 μM)-induced transient (C) and sustained (D) currents in DRG neurons from colitis rats. (E) Diagram showing the effect of progesterone on ATP-induced transient and sustained inward currents in DRG neurons from colitis rats. **p < 0.01.
3. Results

3.1. Differences of VMR among sham-op, ovariectomized, estrogen-treated and estrogen and progesterone-treated colitis rats

The magnitude of VMR was measured when applying colonic distension pressures of 20, 40, 60 and 80 mmHg. Compared with that in the sham-op colitis rats, the magnitude of the VMR in the ovariectomized colitis (OVX) rats was significantly decreased \((p = 0.007, \text{Fig. 1A})\). Estrogen produced a significant facilitation of the VMR \((p = 0.002, \text{Fig. 1A})\) compared with that in the ovariectomized colitis rats. There was no difference between ovariectomized and estrogen–progesterone-treated groups \((p = 0.143, \text{Fig. 1A})\).

3.2. Colon compliance in sham-op, ovariectomized and estrogen–treated colitis rats

Colonic compliance was measured in sham, OVX, and estrogen-treated rats after colitis. There was no difference in the compliance of the colon in the above three groups \((p > 0.05, \text{Fig. 1B})\). The volume–pressure curves were identical \((\text{Fig. 1B})\). The linear relationship between the volume and resulting pressure in groups indicates that the balloon was compliant over this volume range, and the pressure readings reflected the pressure exerted by the colon wall on the balloon.

3.3. Changes in P2X3 and P2X2 mRNA expression in DRG among sham, OVX, estrogen-treated and estrogen–progesterone-treated colitis rats

After the colon was inflamed by TNBS, P2X3 receptor mRNA in the ovariectomized rats, compared with the sham-op colitis rats, was significantly decreased in DRG \((p < 0.05, \text{Fig. 2A})\). Estrogen significantly increased P2X3 mRNA expression compared with that in ovariectomized rats \((p < 0.05, \text{Fig. 2A})\). While in the estrogen–progesterone-treated group, the P2X3 level was similar to that in the ovariectomized group \((p > 0.05, \text{Fig. 2A})\). The P2X3 mRNA expression in DRG did not change significantly among four groups \((p > 0.05, \text{Fig. 2B})\).

3.4. Modulatory effect by estradiol and progesterone on the P2X3 receptor-induced inward currents in cultured DRG neurons from colitis rats

In the present study, we found that in 65% isolated lumbar and sacral dorsal root ganglion neurons, 17β-estradiol \((0.001–1 \mu \text{M})\) rapidly inhibited P2X3 receptor-induced transient currents to \(44 \pm 5.8\%\) of controls, which could be blocked by ICI 182, 780, 80, an antagonist of estrogen receptors \([37]\). In the present study, we further compared the rapid modulation of estrogen on ATP-induced transient currents in dorsal root ganglion neurons from control and colitis female rats with intact ovaries. 151 and 148 neurons on total from two groups were investigated, respectively. The results showed that from 114 control neurons responded to ATP \((100 \mu \text{M})\) with a transient current, \(71 \pm 62\%\) was inhibited to \(61 \pm 6.3\%\) of controls by 4 min application of 17β-estradiol \((1 \mu \text{M})\). While the same agonist evoked sustained currents were not affected \((n = 37; \text{Fig. 3B})\). Similarly, in the neurons from colitis rats, 73 from 122 \((60\%)\) of which responded to ATP \((100 \mu \text{M})\) with transient currents, was inhibited by 17β-estradiol in the same concentration to \(54 \pm 6.7\%\) of controls. Whereas in a subpopulation \((15\%, 18/122)\) of cells, estradiol potentiated the transient current induced by ATP \((100 \mu \text{M})\) to 336 ± 45% of the controls \((p < 0.05; \text{Fig. 3C and D})\), and in \(31\% (8/26)\) of neurons, sustained currents evoked by ATP \((100 \mu \text{M})\) was also potentiated to 122 ± 12% of the controls \((p < 0.05; \text{Fig. 3D and E})\), the potentiation of both transient or sustained currents was blocked by ICI 182, 780 \((1 \mu \text{M})\), an estradiol receptor \((\alpha \text{ and } \beta)\) antagonist \((\text{Fig. 3C–E})\).

Similar experiments were carried out to observe the effect of progesterone on the P2X3-mediated transient currents. In 115 neurons from control female rats, 109 \((94.8\%)\) showed a rapid inhibition by progesterone \((0.1 \mu \text{M})\) to 13.4 ± 2.9% of control \((\text{Fig. 4A and B})\), and in 30 DRG neurons from colitis female rats, 11 \((37\%)\) showed a rapid inhibition by progesterone \((1 \mu \text{M}; \text{Fig. 4C and E})\) to 67 ± 10% of control, while none of them showed the rapid potentiation as seen with estrogen. Progesterone had no effect on the sustained currents induced by ATP in the neurons from either control \((n = 39; \text{Fig. 4B})\) or colitis female rats \((n = 27; \text{Fig. 4D and E})\).

4. Discussion

The VMR is a contraction of the abdominal muscles in response to colorectal distension, which provides a reliable noxious visceral stimulus in awake rats that is reproducible and produces measurable, reliable, physiological and behavioral responses \([41]\). In the present study, we first found that VMR was significantly decreased in OVX rats after colitis compared with that observed in sham-op rats after colitis, which was reversed after estrogen treatment. These data demonstrate that the reduced visceral sensitization and colon activity was probably related with the significantly decreased estrogen level in inflamed conditions. VMR results in this study are consistent with previous studies in normal rats, for example, ovariectomy decreased the VMR and that estrogen replacement reversed the decrease \([42,43]\). Rats in proestrus exhibited more sensitivity to colonic balloon distension than in other phases \([40,44]\). These results provide evidence for exogenous and endogenous estrogen modulation of visceral nociceptive processing that could contribute to sex differences in both physiological and pathological conditions.

In the early 1970s’ oral contraceptives were identified as a risk factor for relapse in IBD or Crohn’s disease \([45]\), and a later report cited female gender as a risk factor for relapse in ulcerative colitis \([24]\). Also, IBS is most prevalent in premenopausal women \([34,46]\). These phenomena suggest the further relation between sex hormones and colon inflammation. One hypothesis is that gonadal hormones increase visceral sensitivity. Primary afferent nerves innervating visceral tissues express P2X2, vanilloid receptor 1 (TRPV1) and N-methyl-D-aspartate receptors (NMDARs) \([47–49]\). One of the mechanisms through which estrogen modulates primary afferent activity is the potentiation of NMDA currents in DRG neurons, increasing their mechanical sensitivity \([50]\), suggesting a modulatory effect by estrogen on pain-related receptors at sensory ganglion level.

Evidence has shown that the excitability of visceral afferent nerves was enhanced following injury or ischemia and during inflammation, for example, in colitis and IBS \([17,18,39]\). Under these conditions, ATP is released from various sources to cause sensitization of afferent nerves to mechanical or chemical stimuli. ATP release and P2X3 and P2X2/3 receptor-mediated nociceptive sensory nerve responses were enhanced in a model of IBD \([39]\) and P2X3 receptor expression was also shown to be enhanced in a model of IBS \([17]\). P2X receptors have been regarded as the potential targets for the drug treatment of IBS \([51]\). It has been suggested that agonists acting on P2X receptors on intrinsic enteric neurons may enhance gastrointestinal propulsion and secretion and that these drugs might be useful for treating constipation–predominant IBS, while P2X antagonists might be useful for treating diarrhea–predominant IBS \([52]\).

In our study, colonic inflammation after ovariectomy significantly decreased the levels of P2X3 receptor mRNA in the dorsal root ganglion compared with that in colitis rats with intact ovaries.
We then isolated the effects of estrogen by ovariectomy, followed by estrogen replacement. Estrogen replacement significantly facilitated the decreased P2X3 mRNA in the ovariectomized colitis rats. P2X2 mRNA in DRG did not change significantly in the groups. These findings suggest that estrogen enhances visceral nociception in the ovariectomized rats with colitis via increasing P2X3 mRNA expression. However, the contribution of progesterone and other associated steroids from intact gonads must also be taken into account. We then found that estrogen–progesterone replacement did not reverse either the VMR or the P2X3 mRNA level compared to intact controls. We then isolated the effects of estrogen by ovariectomy, followed by estrogen replacement. Estrogen replacement significantly facilitated the decreased P2X3 mRNA in the ovariectomized colitis rats. Combined with the estrogen effects on VMR and P2X3 mRNA level, clear that the transient current and the transient component of the P2X3 receptors are mediated by homomorphic P2X2 receptor. The sustained current and the sustained component of the biphasic current were attributable to the activation of heteromeric P2X2/3 receptors [37]. When using patch-clamp recording techniques in this study, we found for the first time, that in colitis rats, estradiol rapidly potentiated the sustained and transient currents evoked by ATP in a subpopulation of DRG neurons, which were blocked by ICI 182,780, an antagonist of the estrogen receptors. These results suggest that estradiol may enhance the P2X3 subunit containing-receptors by non-genomic mechanisms. Although there was no direct evidence for an effect by estradiol on primary afferent neurons innervating inflamed colorectum, a similar phenomenon (P2X2 receptor-mediated currents were enhanced by estrogen at 1 μM) was not observed in control rats. Further, in the control or colitis female rat DRG neurons, we did not find any potentiation by progesterone in P2X3 subunit containing-receptor-mediated currents. These results suggest that estrogen, but not progesterone, may increase the cell sensitivity to acute nocuous and noxious stimuli when the primary sensory endings have been inflamed. Combined with the estrogen effects on VMR and P2X3 mRNA level, these results are consistent with the important observation that women are more prone to IBS.[34,46]. In conclusion, P2X3 receptors appear to be important contributors to altered colon functions in colitis rats with relation to endogenous estrogen modulation, thus they might be regarded as potential targets for the drug treatment of IBS.

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References


