Colonic Inflammation Increases the Contribution of Muscarinic M₂ Receptors to Carbachol-Induced Contraction of the Rat Colon

Dina M. Jragh^a Islam Khan^b Mabayoje A. Oriowo^a

Departments of ^aPharmacology and Toxicology, and ^bBiochemistry, Faculty of Medicine, Kuwait University, Jabriya, Kuwait

Key Words

Muscarinic receptors • Carbachol • Pirenzepine • Methoctramine • 4-Diphenylacetoxy-N-methyl piperidine • Trinitrobenzene sulfonic acid

Abstract

Objectives: Carbachol-induced contraction of the rat colon is impaired in rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis. The main objective of this study was to examine the effect of colitis on the expression and function of muscarinic (M) receptor subtypes in the rat colon. **Materials and Methods:** Rats (n = 80) were treated with TNBS and used 5 days later for measurement of contractility, myeloperoxidase activity, histology and expression of muscarinic receptor isoforms using Western blot analysis. **Results:** Carbachol produced concentration-dependent contractions of colonic segments from control (n = 40) and TNBS-treated (n = 40) rats with no significant difference in potency. However, the maximum response to carbachol was significantly reduced in colon segments of TNBS-treated rats. The selective muscarinic receptor antagonists 4-diphenylacetoxy-N-methyl piperidine (4-DAMP, M₃), pirenzepine (M₁) and methoctramine (M₂) antagonized carbachol-induced contraction in control (9.1 ± 0.1, 6.7 ± 0.3 and 6.0 ± 0.1, respectively) and TNBS-treated rats (9.2 ± 0.2, 6.9 ± 0.2, 6.7 ± 0.2). The –logK_B values in control rats are consistent with an action of

carbachol on muscarinic M₃ receptors. There was no significant difference in –logK_B values for 4-DAMP and pirenzepine in control and TNBS-treated rats, but methoctramine was fivefold more potent in TNBS-treated rats, possibly indicating an increased contribution of muscarinic M₂ receptors to carbachol-induced contraction in the inflamed colon. The expression of M₂ receptors was also significantly increased in colon segments from TNBS-treated rats, confirming the increased role of muscarinic M₂ receptors in the inflamed colon. **Conclusions:** The data show that while only M₃ receptors appeared to mediate carbachol-induced contraction in control segments, expression of both M₂ and M₃ receptors was increased in the inflamed rat colon.

Copyright © 2011 S. Karger AG, Basel

Introduction

Impaired carbachol-induced contraction of colon strips from 2,4,6-trinitrobenzene sulfonic acid (TNBS)-treated rats has been reported. Muscarinic receptors belong to the group of G-protein-coupled seven-transmembrane domain receptors. Muscarinic receptors are not homogeneous as five subtypes (M₁–M₅) have been identified [1]. All these subtypes are expressed in the gastrointestinal tract [2, 3]. Stimulation of M₃ receptors is associated with activation of phospholipase C with the subse-

quent hydrolysis of membrane phosphoinositol leading to the generation of inositol-1,4,5-triphosphate and diacylglycerol. Inositol-1,4,5-triphosphate releases Ca^{2+} from intracellular stores, while diacylglycerol activates protein kinase C. Activation of M_2 receptor, however, is negatively coupled to cAMP. It has been reported in several studies that the M_3 -subtype is predominantly involved in smooth muscle contractions induced by muscarinic receptor agonists [1]. However, some studies have reported M_2 receptor-mediated contractions in cat [4] and human [5] esophageal smooth muscle cells. In addition, a role for M_2 receptors can be revealed under some conditions involving either inactivation of M_3 receptors or accumulation of cAMP in the tissues since M_2 receptors are negatively coupled to adenylyl cyclase [6, 7].

It has been reported that pathological states can alter the expression and function of muscarinic receptor subtypes. For example, an increased contribution of M_2 receptors to carbachol-induced contractions in urinary bladder strips from rats with experimentally induced overactivity of the bladder has been reported [8]. A similar finding has been reported in inflamed canine ileum [9–11] and cat esophagus [12]. There is no information about the effect of experimental colitis on the expression and function of muscarinic receptor subtypes in the rat colon. Therefore, the main objective of this study was to examine the effect of TNBS-induced colitis on the expression and function of muscarinic receptor subtypes in the rat colon.

Materials and Methods

Male Sprague-Dawley rats ($n = 80$, 40 each group, 150 and 250 g) were used in this study. The animals were kept in a controlled environment and provided with standard rodent chow (Special Diet Services, England) and free access to water. All the animals were bred and maintained in the Animal Resource Center, Faculty of Medicine, Kuwait University, Kuwait.

Induction of Colitis

Colitis was induced by colonic instillation of 30 mg TNBS in 0.5 ml ethanol under light ether anesthesia. Control rats ($n = 40$) received an equivalent amount of the solvent. The animals were used 5 days after the injection of TNBS. Control and TNBS-treated rats ($n = 40$ each) were weighed before and after the induction of colitis.

Myeloperoxidase Assay

On the 5th day after TNBS administration, the rats were sacrificed and about 2.5 cm of the terminal colon was isolated and used for the assay of myeloperoxidase (MPO) activity, a marker of neutrophil infiltration into the site of inflammation. Briefly, co-

lon segments were finely minced and homogenized in 5 ml of 14 mM hexadecyltrimethylammonium bromide (Sigma) containing 50 mM potassium phosphate buffer (pH 6.0), with a polytron (Janke & Kunkel, Germany). The supernatants were collected by centrifugation and used to estimate MPO activity as previously described [13]. Enzyme activity was expressed as units per milligram of tissue. Enzyme unit is defined as micromoles of H_2O_2 converted into product per minute per milligram of tissue at room temperature.

Contractility Experiments

Approximately 2.0-cm segments of the colon (with the mucosa intact) were cut and set up for isometric longitudinal smooth muscle contraction recording in 25.0-ml organ baths containing Krebs solution of the following composition (mM): 119 NaCl, 4.7 KCl, 2.5 CaCl_2 , 1.2 MgSO_4 , 1.2 KH_2PO_4 , 25 NaHCO_3 , and 11 glucose (pH = 7.4). The solution was gassed continuously with 95% O_2 , 5% CO_2 mixture and the temperature was maintained at 37°C. Isometric responses were recorded through UF1 dynamometer on a Lectromed four-channel polygraph (Multitrace 4P). A resting tension of 1 g was applied and the tissues were allowed to stabilize for 30 min during which the bath fluid was changed at least once. After the period of equilibration, 80 mM KCl was added to the bath to test for tissue viability.

Characterization of Muscarinic Receptor Subtypes Mediating Carbachol-Induced Contraction

In order to investigate the role of muscarinic receptor subtypes in carbachol-induced contractions, the effects of selective muscarinic receptor subtype antagonists on carbachol-induced contractions were examined. In this series of experiments, increasing concentrations of carbachol were added cumulatively to generate a concentration-response curve in the absence and also in the presence of selective muscarinic receptor subtype antagonists; only one concentration of each antagonist was tested on each preparation. Briefly, after establishing the control concentration-response curve for carbachol (added cumulatively) in tissues obtained from control and TNBS-treated rats, antagonists were added to the bath and allowed to equilibrate with the tissues for 30 min before reestablishing agonist concentration-response curve. The EC_{50} values (concentration of the agonist required to give 50% of the maximum response) were used to calculate concentration ratios (CRs). Antagonist potency was expressed as the $-\log K_B$ where K_B was calculated using the equation: $K_B = (\text{antagonist concentration})/\text{CR} - 1$.

Expression of Muscarinic Receptor Subtypes in the Colon

Colonic tissues from control and treated animals were removed and cleaned carefully with ice-cold phosphate-buffered saline. Tissue segments were weighed and used immediately or frozen in liquid nitrogen and stored at -80°C until use. Tissue samples were minced with scissors and homogenized with a polytron (Brinkman Instruments Co., Westbury, N.Y., USA) in 10 ml of ice-cold 3-(N-morpholino)propanesulfonic acid (MOPS)-sucrose buffer, pH 7.4, containing (in mmol/l) 20 MOPS, 300 sucrose and 20 EDTA. The lysates were centrifuged at 1,620 g (Beckman) for 10 min and the supernatants centrifuged at 10,000 g for 10 min (Beckman, JA-20). The supernatants were collected and centrifuged at 188,000 g for 45 min (Beckman Instruments, Palo Alto, Calif., USA). The microsome pellets so obtained were ho-

Fig. 1. Graph showing MPO activity in colon segments from control (C) and TNBS-treated (T) rats.

mogenized using ice-cold MOPS-sucrose buffer with a tissue homogenizer (Eberbach Corp., Mich., USA) and designated as crude microsomes.

Western Blot Analysis

To measure the expression of muscarinic receptor subtypes in the colon, aliquots of crude microsomes were separated on an 8% polyacrylamide gel and were then transferred electrophoretically to a nitrocellulose membrane (BioRad) overnight. After blocking with 5% non-fat milk solution in phosphate-buffered saline, the nitrocellulose membranes were incubated with monoclonal antibodies selective for the different muscarinic receptor subtypes (Santa Cruz Biotechnology, USA) for 3 h. Specific protein bands were developed using suitable secondary antibody conjugated with horseradish peroxidase (Sigma). The nitrocellulose membranes were washed thoroughly and specific bands were developed using a commercial enhanced chemiluminescence reagent kit (Amersham). Band density was estimated using a densitometer (SynGene, Chemi Genius Bio Imaging System). The expression of muscarinic receptor subtypes in colitic tissues was expressed as a percentage of the level in noncolitic controls.

Drug Solutions

The following drugs were used in this study: carbachol hydrochloride, pirenzepine, methoctramine and 4-diphenylacetoxy-N-methylpiperidine methiodide (4-DAMP) obtained from Sigma Chemicals (USA). All the compounds were dissolved in distilled water.

Statistical Analysis

Data were analyzed using Graphpad Prism Software. Data were expressed as mean $\pm$ SE of *n* experiments where *n* is the number of animals used. Differences between mean values were tested for significance using paired or unpaired Student's *t* test (as appropriate). The difference was considered significant when $p < 0.05$.

Fig. 2. Concentration-response curves to carbachol in colon segments from control (■) and TNBS-treated (●) rats. The maximum response in the two groups is shown in the inset.

Results

MPO Activity

As shown in figure 1, MPO activity was significantly higher ($p < 0.05$) in colonic segments from TNBS-treated rats (23.45 ± 7.14 units/min/mg tissue, $n = 5$) compared to segments from control rats (0.46 ± 0.09 units/min/mg tissue, $n = 5$), confirming inflammation in the TNBS-treated rats used in this study.

Contractile Response to Carbachol in Rat Colon

Colon strips developed spontaneous rhythmic contractions within 30 min after they were set up. These rhythmic contractions were maintained throughout the whole experiment (4 h). However, the amplitude of spontaneous rhythmic contractions was greater in colon segments from control rats when compared with preparations from TNBS-treated rats. In addition, the basal tone on the tissues was also maintained throughout the duration of the experiments.

Carbachol (0.1 – $100 \mu\text{M}$) produced concentration-dependent contractions of colon segments from control and TNBS-treated rats. The concentration-response curves to carbachol in colon segments from control and TNBS-treated rats are shown in figure 2. In these curves, response to all concentrations of carbachol in any particular preparation was expressed as a percentage of the maximum response produced by carbachol in that preparation. The pD_2 values were 5.9 ± 0.1 ($n = 4$) and 6.0 ± 0.3 ($n = 3$) in colon segments from control and TNBS-

Fig. 3. Effect of 4-DAMP (3×10^{-8} M) on carbachol-induced contraction in colon segments from control (a) and TNBS-treated (b) rats: contraction in the absence (■) and presence (●, ▲) of 4-DAMP. Each point on the graph is the mean $\pm$ SE of 5 experiments.

treated rats, respectively. These values were not significantly ($p > 0.05$) different from each other. The maximum response to carbachol was significantly reduced in colon segments from TNBS-treated (4.4 ± 0.5 mg/mg tissue weight compared with 17.8 ± 1.6 mg/mg tissue weight in control rats) rats (fig. 2).

Effect of Muscarinic Receptor Antagonists on Carbachol-Induced Contraction

Specifically, the effects of pirenzepine (M_1), methoctramine (M_2) and 4-DAMP (M_3) on carbachol-induced contractions were examined. As shown in figure 3, 4-DAMP (30 nM) produced a parallel rightward shift of carbachol concentration-response curve in colon segments from control and TNBS-treated rats. No suppression of the maximum response to carbachol was observed in any of the groups. The $-\log K_B$ values were 9.1 ± 0.1 ($n = 6$) and 9.2 ± 0.2 ($n = 6$) in colon segments from control and TNBS-treated rats, respectively. These values were not significantly different ($p > 0.05$) from each other.

Pirenzepine ($3 \mu\text{M}$) also produced a parallel rightward shift of the carbachol concentration-response curve in colon segments from control and TNBS-treated rats (fig. 4) with no suppression of the maximum response to carbachol. The $-\log K_B$ values were 6.7 ± 0.3 ($n = 4$) and 6.9 ± 0.2 ($n = 6$) in colon segments from control and TNBS-treated rats, respectively. These values were not significantly different ($p > 0.05$) from each other.

Methoctramine ($3 \mu\text{M}$) also produced a parallel rightward shift of the carbachol concentration-response curve in colon segments from control and TNBS-treated rats (fig. 5) with no suppression of the maximum response to carbachol. The $-\log K_B$ values were 6.0 ± 0.1 ($n = 4$) and 6.7 ± 0.2 ($n = 6$) in colon segments from control and TNBS-treated rats, respectively. These values were significantly different ($p < 0.05$) from each other. Methoctramine was approximately fivefold more potent against carbachol-induced contraction of colon segments from TNBS-treated rats.

Expression of Muscarinic Receptor Subtypes in the Colon

As shown in figures 6–8, all muscarinic receptor subtypes (M_1 , M_2 and M_3) were expressed in colon segments from control and TNBS-treated rats. The expression of M_2 and M_3 receptor subtypes was significantly increased in colon segments from TNBS-treated rats, while the expression of M_1 receptors was decreased.

Discussion

A significant increase in MPO activity in colon segments from rats treated with TNBS confirmed colonic inflammation in these rats and is in agreement with previous studies [13–16]. Impaired agonist-induced contraction of colon strips is an established characteristic of coli-

Fig. 4. Effect of pirenzepine (3×10^{-6} M) on carbachol-induced contraction in colon segments from control (a) and TNBS-treated (b) rats: contraction in the absence (■) and presence (●, ▲) of pirenzepine. Each point on the graph is the mean $\pm$ SE of 5 experiments.

Fig. 5. Effect of methoctramine (3×10^{-6} M) on carbachol-induced contraction in colon segments from control (a) and TNBS-treated (b) rats: contraction in the absence (■) and presence (▲) of methoctramine. Each point on the graph is the mean $\pm$ SE of 5 experiments.

tis in humans and experimental animals. Previous studies have shown that contractility of the colon segments to carbachol was depressed in rats treated with TNBS [14, 17–22]. This has been confirmed in this study. The results obtained in this study showed that carbachol induced reproducible and concentration-dependent contraction of colon segments from control and TNBS-treated rats. There was no significant difference in pD_2 values for car-

bachol between the two groups. This is in agreement with previous reports [14]. However, there was a significant reduction in the maximum response to carbachol in colon segments from TNBS-treated rats, confirming previous observations in this tissue [14, 17–22].

Carbachol-induced contractions of the rat colon are mediated via direct activation of muscarinic receptors since these contractions are not affected by tetrodotoxin

Fig. 6. Expression of muscarinic M_1 receptor in colon segments from control (C) and TNBS-treated (T) rats. **a** Representative gel picture showing the expression of muscarinic M_1 receptor in colon segments from control and TNBS-treated rats. **b** Bar chart showing the mean of 3 experiments.

Fig. 7. Expression of muscarinic M_2 receptor in colon segments from control (C) and TNBS-treated (T) rats. **a** Representative gel picture showing the expression of muscarinic M_2 receptor in colon segments from control and TNBS-treated rats. **b** Bar chart showing the mean of 3 experiments.

Fig. 8. Expression of muscarinic M_3 receptor in colon segments from control (C) and TNBS-treated (T) rats. **a** Representative gel picture showing the expression of muscarinic M_3 receptor in colon segments from control and TNBS-treated rats. **b** Bar chart showing the mean of 3 experiments.

but are abolished by atropine, a nonselective muscarinic receptor antagonist. Five subtypes of muscarinic receptors, M_1 , M_2 , M_3 , M_4 and M_5 , have been identified. These subtypes are expressed in the gastrointestinal tract [2, 3]. M_3 muscarinic receptors mediate the contractile response to carbachol and related agonists, while M_2 -receptor-mediated contractions have been demonstrated in the guinea pig colon under conditions in which levels of cAMP have been elevated or M_3 receptors have been inactivated [6, 7]. The role of these muscarinic receptor subtypes in carbachol-induced contraction in control and TNBS-treated rats was investigated using selective subtype antagonists. The focus of this study was on M_1 , M_2 , M_3 subtypes since previous studies have reported very low (or no) levels of expression of M_4 and M_5 muscarinic receptor subtypes in gastrointestinal tract smooth muscles. The results showed that 4-DAMP produced a parallel rightward shift of the concentration-response curve to carbachol in colon segments from control rats with no reduction in the maximum response to carbachol. The pK_B value obtained for 4-DAMP (9.1 ± 0.1) is consistent with an action on muscarinic M_3 receptors confirming a role for the M_3 receptor subtype in carbachol-induced contractions of the rat colon similar to previous reports of the guinea pig colon [6, 7]. M_3 receptors have also been shown to mediate contraction in other tissues including esophagus, airway smooth muscle, urinary bladder and ileum [1, 8, 10, 23–25]. Even though pirenzepine and methoctra-

mine also produced a parallel rightward shift of carbachol concentration-response curves (also with no reduction in the maximum response), the pK_B values, 6.7 ± 0.3 and 6.0 ± 0.1 for pirenzepine and methoctramine, respectively, were significantly lower than expected for a response mediated via M_1 and M_2 receptor subtypes, but were typical of values for an action of these antagonists on M_3 receptors [1, 8, 10, 23–25], indicating that M_1 and M_2 receptors are not involved in carbachol-induced contraction of colon segments from control rats and therefore confirming that these contractions were mediated via M_3 receptors. In colon segments from TNBS-treated rats, 4-DAMP also produced a parallel rightward shift of the carbachol concentration-response curve without reducing the maximum response to carbachol. The pK_B value was calculated to be 4-DAMP (9.2 ± 0.1) and this was not significantly different from the corresponding value in colon segments from control rats. This would suggest that TNBS-induced colitis did not produce a change in M_3 receptor affinity/function of the colon segments. This result contrasts with that of Wells and Blennerhassett [26], who reported diminished potency of 4-DAMP in reducing acetylcholine-induced shortening of isolated smooth muscle cells from rats treated with TNBS. The reason for the difference in the potency of 4-DAMP is not known. It could, however, be due to differences in the type of preparation used – whole longitudinal colon segment (this study) versus dispersed circular

smooth muscle cells [26]. This result also contrasts with that of Giglio et al. [27], who reported diminished potency of 4-DAMP in inhibiting carbachol-induced contraction of urinary bladder smooth muscle from rats with cyclophosphamide-induced cystitis. The result is, however, similar to that of Shi and Sarna [10] in ileal segments from dogs with acetic acid-induced inflammation of the ileum. Even though pirenzepine also produced a parallel rightward shift of the carbachol concentration-response curve without reducing the maximum response to carbachol, the pK_B value (6.9 ± 0.1) was within the range expected for an action on M_3 receptors, and was not significantly different from the corresponding value in colon segments from control rats. This would suggest that M_1 receptors are not involved in carbachol-induced contraction of the rat colon with or without TNBS-induced colitis. Like 4-DAMP and pirenzepine, methoctramine also produced a parallel rightward shift of the carbachol concentration-response curve in colon segments from TNBS-treated rats without reducing the maximum response to carbachol. In contrast to 4-DAMP and pirenzepine, methoctramine was about fivefold more potent (pK_B value of 6.7 ± 0.1 vs. 6.0 ± 0.1 in controls) against carbachol-induced contraction of colon segments from TNBS-treated rats compared with the controls. An increased potency of methoctramine, interpreted as an increased role for muscarinic M_2 receptors, has been reported by Wells and Blennerhassett [26] in dispersed circular smooth muscle cells of the rat colon and by Jadcherla [28] in canine colonic smooth muscle cells. Shi and Sarna [10] also made a similar observation in ileal segments from dogs with acetic acid-induced inflammation of the ileum. An increased effectiveness of methoctramine has also been reported by Sohn et al. [12] in esophageal smooth muscle of the cat. However, the pK_B value (6.7 ± 0.1) obtained in our study, though higher than the corresponding value in colon segments from control rats, was about midway between the affinities of methoctramine for M_2 and M_3 receptors, suggesting an increased contribution of muscarinic M_2 receptors in carbachol-induced contraction and not a switch from M_3 receptors to M_2 receptors mediating the contractions. An increase in the pK_B value for methoctramine coupled with a decrease in the pK_B value for 4-DAMP would suggest a switch from M_3 receptors to M_2 receptors in TNBS-treated rats.

In an attempt to relate the increase in potency of methoctramine in colon segments from TNBS-treated rats with any possible change in the density of muscarinic M_2 receptor subtype, the expression of M_2 receptor

protein was examined. The expression of M_1 and M_3 receptors was also studied for comparison. The results showed that all three receptor subtypes were expressed in colon segments from control and TNBS-treated rats. However, the expression of muscarinic M_1 receptors was significantly reduced in colon segments from rats treated with TNBS, whereas the expression of muscarinic M_2 and M_3 receptors was increased in the same animals. So far, only one study reported in the literature has examined the effect of inflammation on the expression of muscarinic receptor subtypes in the gastrointestinal tract. In that study by Shi and Sarna [11], the expression of neither M_2 nor M_3 receptors was altered by acetic acid-induced inflammation despite the increased potency of methoctramine, a selective M_2 receptor antagonist. However, an increased expression of M_2 receptors has been reported in other pathological states such as hypertrophic denervated bladder [29]. Thus, our observation of an increased expression of M_2 receptors together with the increased potency of methoctramine would suggest an increased role for muscarinic M_2 receptors in carbachol-induced contractions of the inflamed rat colon. We cannot explain the increase in the expression of M_3 receptors observed in this study, but we could speculate that this might have buffered the increased potency of methoctramine and this would explain why the potency of methoctramine, though increased, was not up to what would have been expected for an action on M_2 receptors.

Conclusion

While only M_3 receptors appeared to mediate carbachol-induced contraction in control colon segments, both M_2 and M_3 receptors were involved in colon segments from TNBS-treated rats. The fact that carbachol-induced contraction in colon segments from TNBS-treated rats was attenuated despite the increase in expression of M_2 and M_3 receptors would suggest that the attenuation could be due to changes in postreceptor mechanisms.

Acknowledgments

This study was supported by funds from the College of Graduate Studies, Kuwait University, Kuwait.

References

- Caulfield MP, Birdsall NJ: International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. *Pharmacol Rev* 1998;50:279–290.
- So I, Yang DK, Kim HJ, Min KW, Kang TM, Kim SJ, Kim KW, Park KH, Jeon J-H, Choi K-H, Kim I-G: Five types of muscarinic receptors are expressed in gastric smooth muscle. *Exp Mol Med* 2003;35:46–52.
- Wang J, Krysiak PS, Laurier LG, Sims SM, Preiksaitis HG: Human esophageal smooth muscle cells express muscarinic receptor subtypes M(1) through M(5). *Am J Physiol* 2000;279:G1059–G1069.
- Biancani P, Sohn UD, Rich HG, Harnett KM, Behar J: Signal transduction pathways in esophageal and lower esophageal sphincter circular muscle. *Am J Med* 1997;103:23S–28S.
- Braverman AS, Miller LS, Vegesna AK, Tiwana MI, Tallarida RJ, Ruggieri MR: Quantitation of the contractile response mediated by two receptors: M₂ and M₃ receptor-mediated contractions of human gastroesophageal smooth muscle. *J Pharmacol Exp Ther* 2009;329:218–224.
- Sawyer GW, Ehlert FJ: Contractile roles of the M₂ and M₃ muscarinic receptors in the guinea pig colon. *J Pharmacol Exp Ther* 1998;284:269–277.
- Sawyer GW, Ehlert FJ: Muscarinic M₃ receptor inactivation reveals a pertussis toxin-sensitive contractile response in the guinea pig colon: evidence for M₂/M₃ receptor interactions. *J Pharmacol Exp Ther* 1999;289:464–476.
- Ruggieri MR, Braverman AS: Regulation of bladder muscarinic receptor subtypes by experimental pathologies. *Auton Autacoid Pharmacol* 2006;26:311–325.
- Shi XZ, Sarna SK: Inflammatory modulation of muscarinic receptor activation in canine ileal circular muscle cells. *Gastroenterology* 1997;112:864–874.
- Shi XZ, Sarna SK: Molecular modification of M₂ and M₃ receptor function in canine ileal inflammation. *Neurogastroenterol Motil* 1999;11:261.
- Shi XZ, Sarna SK: G protein-mediated dysfunction of excitation-contraction coupling in ileal inflammation. *Am J Physiol* 2004;286:G899–G905.
- Sohn UD, Harnett KM, Cao W, Rich H, Kim W, Behar J, Biancani P: Acute experimental esophagitis activates a second signal transduction pathway in cat smooth muscle from the lower esophageal sphincter. *J Pharmacol Exp Ther* 1997;283:1293–1304.
- Khan I, Al-Awadi FM: Colonic muscle enhances the production of interleukin-1 β messenger RNA in experimental colitis. *Gut* 1997;40:307–312.
- Khan I, Oriowo MA, Anim JT: Amelioration of experimental colitis by Na-H exchanger-1 inhibitor amiloride is associated with reversal of IL-1 β and ERK mitogen-activated protein kinase. *Scand J Gastroenterol* 2005;40:578–585.
- Wang H, Ouyang Q, Hu RW: Establishment of a trinitrobenzene sulfonic acid-induced rat colitis model. *Chin J Dig Dis* 2002;3:13–17.
- Centinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA: Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. *Tohoku J Exp Med* 2005;206:131–139.
- Snape WJ Jr, Kao HW: Role of inflammatory mediators in colonic smooth muscle function in ulcerative colitis. *Dig Dis Sci* 1988;33:65S–70S.
- Grossi L, McHugh K, Collins SM: On the specificity of altered muscle function in experimental colitis in rats. *Gastroenterology* 1993;104:1049–1056.
- Myers BM, Martin JS, Dempsey DT, Parkman HP, Thomas RM, Ryan JP: Acute experimental colitis decreases colonic circular smooth muscle contractility in rats. *Am J Physiol* 1997;273:G928–936.
- Hosseini JM, Goldhill JM, Bossone C, Pineiro-Carrero V, Shea-Donohue T: Progressive alterations in circular smooth muscle contractility in TNBS-induced colitis in rats. *Neurogastroenterol Motil* 1999;11:347–356.
- Shi XZ, Sarna SK: Impairment of Ca²⁺ mobilization in circular muscle cells of the inflamed colon. *Am J Physiol* 2000;278:G234–G242.
- Kinoshita K, Sato K, Hori M, Ozaki H, Karaki H: Decrease in activity of smooth muscle L-type Ca²⁺ channels and its reversal by NF- κ B inhibitors in Crohn's colitis model. *Am J Physiol* 2003;285:G483–G493.
- Fryer AD, Jacoby DB: Muscarinic receptors and control of airway smooth muscle. *Am J Respir Crit Care Med* 1998;158:S154–160.
- Preiksaitis HG, Krysiak PS, Chrones T, Rajgopal V, Laurier LG: Pharmacological and molecular characterization of muscarinic receptor subtypes in human esophageal smooth muscle. *J Pharmacol Exp Ther* 2000;295:879–888.
- Choppin A, Eglen RM: Pharmacological characterization of muscarinic receptors in mouse isolated urinary bladder smooth muscle. *Br J Pharmacol* 2001;133:1035–1040.
- Wells RW, Blennerhassett MG: Divergent changes to muscarinic and serotonergic signalling following colitis. *Gut* 2005;54:1699–1706.
- Giglio D, Ryberg AT, To K, Delbro DS, Tobin G: Altered muscarinic receptor subtype expression and functional responses in cyclophosphamide induced cystitis in rats. *Auton Neurosci* 2005;122:9–20.
- Jadcherla SR: Inflammation inhibits muscarinic signaling in in-vivo canine colonic circular smooth muscle cells. *Pediatr Res* 2002;52:756–762.
- Braverman AS, Luthin GR, Ruggieri MR: M₂ muscarinic receptor contributes to contraction of the denervated rat urinary bladder. *Am J Physiol* 1998;275:R1654–1660.