

Effect of pinaverium bromide on stress-induced colonic smooth muscle contractility disorder in rats

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Abstract

AIM: To investigate the effect of pinaverium bromide, a L-type calcium channel blocker with selectivity for the gastrointestinal tract on contractile activity of colonic circular smooth muscle in normal or cold-restraint stressed rats and its possible mechanism.

METHODS: Cold-restraint stress was conducted on rats to increase fecal pellets output. Each isolated colonic circular muscle strip was suspended in a tissue chamber containing warm oxygenated Tyrode-Ringer solution. The contractile response to ACh or KCl was measured isometrically on ink-writing recorder. Incubated muscle in different concentrations of pinaverium and the effects of pinaverium were investigated on ACh or KCl-induced contraction. Colon smooth muscle cells were cultured from rats and $[Ca^{2+}]_i$ was measured in cell suspension using the Ca^{2+} fluorescent dye fura-2/AM.

RESULTS: During stress, rats fecal pellet output increased 61 % ($P < 0.01$). Stimulated with ACh or KCl, the muscle contractility was higher in stress than that in control. Pinaverium inhibited the increment of $[Ca^{2+}]_i$ and the muscle contraction in response to ACh or KCl in a dose dependent manner. A significant inhibition of pinaverium to ACh or KCl induced $[Ca^{2+}]_i$ increment was observed at 10^{-6} mol/L. The IC_{50} values for inhibition of ACh induced contraction for the stress and control group were 1.66×10^{-6} mol/L and 0.91×10^{-6} mol/L, respectively. The IC_{50} values for inhibition of KCl induced contraction for the stress and control group were 8.13×10^{-7} mol/L and 3.80×10^{-7} mol/L, respectively.

CONCLUSION: Increase in $[Ca^{2+}]_i$ of smooth muscle cells is directly related to the generation of contraction force in colon. L-type Ca^{2+} channels represent the main route of Ca^{2+} entry. Pinaverium inhibits the calcium influx through L-type channels; decreases the contractile response to many kinds of agonists and regulates the stress-induced colon hypermotility.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional

gastrointestinal disorder in which abdominal pain is associated with changes of bowel habit and abdominal distension^[1,35]. The pathophysiological mechanisms for IBS are not clear and therefore the therapy is usually empirical. Abnormal contraction of intestinal smooth muscle may be important in producing the main IBS symptoms^[2-5,43], thus, modifying the contractility is often the major aim in the treatment of IBS^[6,7]. Pinaverium bromide, a L-type calcium channel blocker with selectivity for the gastrointestinal tract, effectively relieves pain, diarrhea and intestinal discomfort, provides good therapeutic efficacies without significant adverse effects on IBS patients^[8-10].

To evaluate the rational use of calcium channel blockers in colonic motor activity affecting the contraction of smooth muscle and to explore the physiopathology in such functional bowel disorders, we conducted cold-restraint stress on rats to induce fecal pellet output increased, investigated changes in contractility of circular muscle isolated from stressed colon treated with or without pinaverium. In addition, to further clarify the mechanism in the action of pinaverium we cultured colonic smooth muscle cells from rats, analyzed the influence of pinaverium on the free cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$). The circular muscle was chosen because it was the musculature predominantly responsible for propulsive contractile activity.

MATERIALS AND METHODS

Animal model

Male Wister rats, weighing 250-300 g, were housed in individual cages and provided with standard rodent chow and tap water for at least one week prior to the experiment. Room temperature was maintained at 25-28 °C. Rats were deprived of food but not water for 24 h before testing. Rats were studied for 2 h in normal living cages at room temperature (control, no stressed animals) or in wire-mesh restraining cylinders (5.5 cm in diameter × 18.5 cm in length) placed in a cold (4-6 °C) environment (cold-restraint stressed animals). The quality of fecal pellets expelled by each animal was measured after 2 h.

Recording of contractile responses to the stimulation of agonist

Each animal was killed by cervical dislocation, and the segments of distal colon (4 cm from anus) were removed. Circular smooth muscle strips (2 mm × 8 mm) were prepared by separating the mucosa and serosa from the muscle layers.

Muscle strips were mounted in individual tissue baths containing warm (37 °C) oxygenated (95 % O_2 and 5 % CO_2) Tyrode-Ringer solution (pH 7.4, composition in mmol/L: 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 11.9 $NaHCO_3$, 0.4 NaH_2PO_4 , 5 Glucose) and attached to an isometric force transducer. The contractile activity was detected as change of tension, which was generated by circular muscle^[39-41]; the contractile response was measured isometrically on ink-writing recorder and the data were expressed as milli-Newton per square millimeter (mN/mm²).

Muscle strips from the control and stressed rats were randomly stimulated by ACh (10^{-5} mol/L) or KCl (60 mmol/L).

To investigate the effect of pinaverium bromide on muscle contractility, each muscle strip was stimulated by ACh or KCl twice before and twice after introduction of pinaverium (10^{-7} - 3×10^{-5} mol/L) following at least a 15 min equilibration period for each concentration of the blocker in Tyrode-Ringer solution. For a single strip, up to three different concentrations of pinaverium were applied cumulatively. Mean values obtained before and after the treatment of the blocker were compared. The contractile response to pinaverium was expressed as the percent of decreased response in the control recorded immediately before exposure to the calcium channel blocker.

Isolation and culture of colonic smooth muscle cells

Smooth muscle cells were isolated from the colon of rats by two consecutive digestions with collagenase respectively, following the method previously described^[11]. Briefly, after peeling off the serosal and mucosal layers, muscle tissues were minced into small pieces of 2 to 3 mm² and incubated in culture medium A (pH 7.4, composition in mmol/L: 25 HEPES, 120 NaCl, 4 KCl, 2 CaCl₂, 0.6 MgSO₄, 2.6 KH₂PO₄ and 14 glucose) containing 0.1 % collagenase, 0.1 % BSA, and 0.01 % soybean trypsin inhibitor for 40 min at 31 °C, the culture medium was filtered through a nylon mesh. The filtrate, which contained isolated cells, was diluted with enzyme-free culture medium A and centrifuged at 150 g for 5 min. The cell pellet was then diluted in PBS. The remaining tissue from the first incubation was re-incubated in culture medium A for 30 min at 31 °C and fragments were dispersed into single cells by passages in and out the inverted wide end of a 5-ml pipette. The acquired cell suspension was filtered through a nylon mesh (500 μm). Isolated cells from the two incubations were pooled and counted. Freshly isolated colonic muscle cells were resuspended in DMEM medium with high glucose (12 mmol/L), sodium pyruvate (1 mmol/L), 10 % heat-inactivated fetal bovine serum and antibiotics (100 UI/mL penicillin G, 100 UI/mL streptomycin, 50 μg/mL gentamicin and 3 μg/mL amphotericin B). Cells were plated in sterile flasks at a density of 5×10^4 cells/mL and incubated at 37 °C in a humidified atmosphere with 5 % CO₂. Culture medium was exchanged every 3 d. Only those cells that were cultured for 14 to 17 days were used for subsequent studies.

Measurement of [Ca²⁺]_i in colonic smooth muscle cells

After the cultured cells were digested with 0.125 % trypsin, [Ca²⁺]_i was measured in cell suspension (10^6 cells/ml) using the Ca²⁺ fluorescent dye fura-2/AM as described by Shi *et al.*^[11]. Muscle cells were suspended in a modified HEPES buffer containing (in mmol/L) 10 HEPES, 125 NaCl, 5 KCl, 1 CaCl₂, 0.5 MgSO₄ and 5 glucose, and were incubated with 5 μmol/L fura-2/AM for 30 min at 37 °C. The fura-2/AM treated samples were diluted, centrifuged twice, and suspended in 1.5 ml solution with the same composition for immediate measurement of [Ca²⁺]_i. Fluorescence was measured at 510nm with excitation wavelengths alternating between 340nm and 380nm. Autofluorescence value of unloaded cells was subtracted from the fluorescence value of fura-2/AM treated cells. The [Ca²⁺]_i was calculated from the fluorescence ratio ($R = F_{340}/F_{380}$), $[Ca^{2+}]_i = K_d \cdot (R - R_{min}) / (R_{max} - R)$. The dissociation constant (K_d) of 224 nmol/L was used for fura-2/AM. The maximum and minimum fluorescence value were determined after adding 10 % Triton-X100 and 5 mmol/L EGTA, respectively, in each sample.

To investigate the effect of pinaverium bromide on [Ca²⁺]_i, cells were incubated for 20 min in HEPES buffer containing pinaverium (10^{-7} - 10^{-5} mol/L) before the stimulation of agonists.

Statistical analysis

All values were expressed as $\bar{x} \pm s$. Statistical analysis was done

by one-way analysis of variance and the Student's *t*-test for unpaired observations. $P < 0.05$ was considered statistically significant.

RESULTS

Defecation during stress

Cold-restraint stress significantly increased fecal pellet output. During 2 h, there were 2.40 ± 1.23 fecal pellets in the stressed rats vs 1.49 ± 1.04 in the control ($n = 45$ each, $P < 0.001$). In addition, stressed rats showed a higher incidence of poorly formed fecal pellets, which appeared to contain more fluid.

After the cold-restraint stress for 2 hours, there was no gastric ulcers in rats, and the histological examination on the colon showed that no inflammatory responses (erosion, ulcer, edema, petechia, *etc.*) were found.

Effect of stress on colonic contractility

In the Tyrode-Ringer solution, ACh or KCl induced colonic circular muscle contraction and produced increase in force. Contractility of muscle in the stress group was significantly higher than that in control group ($P < 0.05$) (Table 1).

Table 1 Contractile response to ACh and KCl in colonic circular muscle (mN/mm², $\bar{x} \pm s$)

	ACh (10^{-5} mol/L)	KCl (60 mmol/L)
Control	71.56 ± 19.36 ($n = 8$)	73.85 ± 13.08 ($n = 9$)
Stress	109.66 ± 34.42^a ($n = 8$)	119.54 ± 19.06^a ($n = 8$)

^a $P < 0.05$, vs control.

Effect of Pinaverium on smooth muscle contractility

Pinaverium bromide inhibited the contractile response to ACh or KCl in the control and stressed colonic circular muscle in a dose dependent manner. The IC₅₀ value for inhibition of 10^{-5} mol/L ACh induced contraction in the stress group was 1.66×10^{-6} mol/L, and in the control was 0.91×10^{-6} mol/L. Effects of pinaverium on stressed colon were not significantly different from that in the control except for the effects of 3×10^{-6} mol/L and 10^{-5} mol/L. The inhibitory response at the maximum concentration of 3×10^{-5} mol/L pinaverium was 99.57 ± 0.62 % ($n = 8$) and 98.24 ± 1.92 % ($n = 7$), respectively, in the stress and the control group there was no significant difference between them ($P > 0.05$). Concentration-response curve was shown in Figure 1.

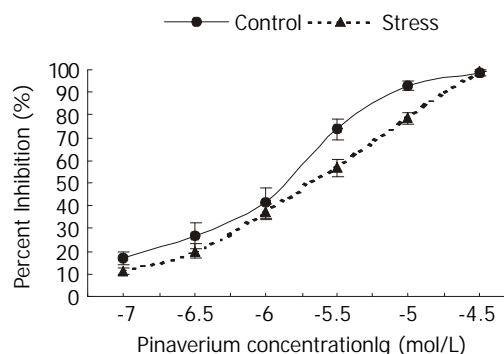


Figure 1 Effect of pinaverium on ACh-induced colonic circular muscle contraction. ^a $P < 0.05$, vs control.

The IC₅₀ values for the inhibition of 60 mmol/L KCl induced contraction in the stress group and control group were 8.13×10^{-7} mol/L, and 3.80×10^{-7} mol/L, respectively. Effects of pinaverium on stressed colon were significantly different from

that in the control at 3×10^{-7} mol/L and 10^{-6} mol/L. The inhibitory response of pinaverium at 3×10^{-5} mol/L was 99.68 ± 0.44 % and 99.54 ± 0.65 %, respectively, in the stress and the control group there was no significant difference between them ($P > 0.05$). It was shown in Figure 2.

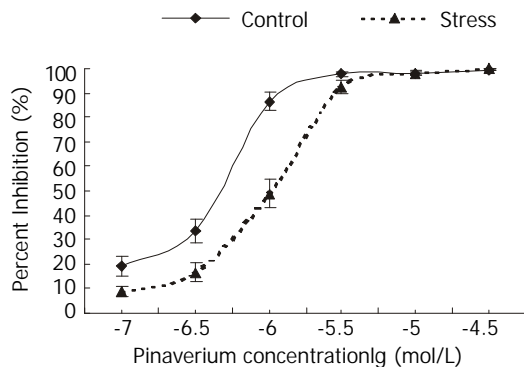


Figure 2 Effect of pinaverium on KCl-induced colonic circular muscle contraction. ^a $P < 0.05$, vs control.

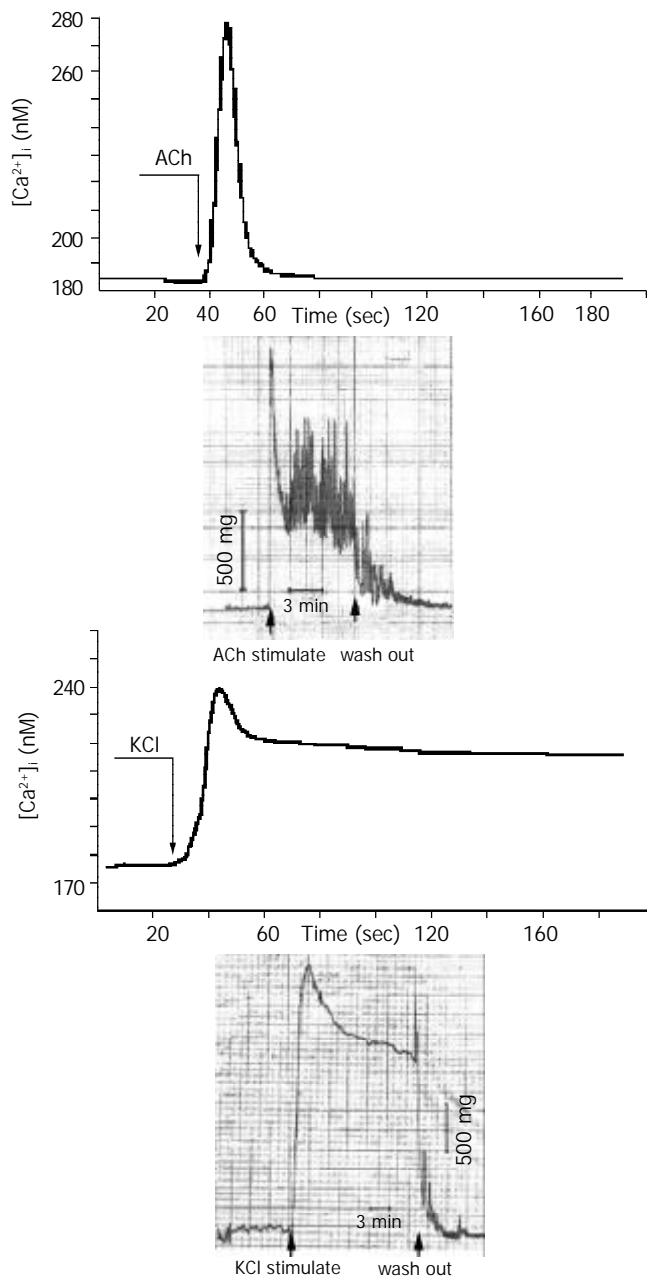


Figure 3 Effect of ACh and KCl on $[Ca^{2+}]_i$ and contraction of colonic smooth muscle.

Effect of Pinaverium on $[Ca^{2+}]_i$

ACh at 10^{-5} mol/L evoked a rapid increase in $[Ca^{2+}]_i$ that reached a peak in 10–15 s (Figure 3A). Similarly, 60 mmol/L KCl increased $[Ca^{2+}]_i$ in colonic muscle cells, the peak $[Ca^{2+}]_i$ was reached at 15–20 s (Figure 3B). Figure 3 also showed that the force of contraction in colonic muscle had no linear relationship with $[Ca^{2+}]_i$, and both velocity and amplitude of $[Ca^{2+}]_i$ increment in contraction were directly related to the force generation.

Pinaverium inhibited the increment of $[Ca^{2+}]_i$ in response to ACh or KCl in a dose dependent manner. A significant inhibition to 10^{-5} mol/L ACh or 60 mM KCl was observed at 10^{-6} mol/L pinaverium, as expressed in Table 2.

Table 2 Effect of pinaverium bromide on the increment of $[Ca^{2+}]_i$ ($\Delta [Ca^{2+}]_i$) in response to ACh and KCl (nmol/L, $\bar{x} \pm s$)

Concentration of pinaverium (mol/L)	ACh (10^{-5} mol/L) (n=7)	KCl (60 mmol/L) (n=6)
0	94.78 ± 15.99	64.61 ± 8.06
10^{-7}	83.57 ± 13.54	55.73 ± 17.39
10^{-6}	34.73 ± 13.98^a	41.71 ± 1.61^a
10^{-5}	27.09 ± 5.64^a	40.34 ± 10.96^a

Figures in this table are the increment of $[Ca^{2+}]_i$ in response to ACh and KCl. $\Delta [Ca^{2+}]_i = [Ca^{2+}]_i \text{After (ACh, KCl) stimulated} - [Ca^{2+}]_i \text{Before (ACh, KCl) stimulated}$. ^a $P < 0.05$, vs pinaverium 0 mmol/L.

DISCUSSION

Different types of stresses play important roles in the onset and modulation of IBS symptoms^[12–18]. Our results showed that cold-restraint stress significantly increased fecal pellet output in rats; which appeared to be the results of an increased colonic motility. There was no gastric ulcer or histological changes on the colon in the model of stressed rats, so it was an appropriate animal model to investigate the functional intestinal dysmotility such as IBS. In vitro studies using colonic smooth muscle strips obtained from rats showed that stress was accompanied by an increase in the contractility of muscle, it may be the reason for the colonic dysmotility.

Abnormal contraction of intestinal smooth muscle may be important in the pathology of IBS symptoms, thus, modifying the contractility is often the major aim in treating IBS^[6,7,19]. A new approach to such disorder is based on the fact that Ca^{2+} is involved in the mechanism of excitation-contraction coupling, which directly or indirectly controls the contractility of smooth muscle^[44]. $[Ca^{2+}]_i$ regulates the contractile state of smooth muscle; increase of $[Ca^{2+}]_i$ is the primary stimulus for contraction^[20–22]. Elevation of $[Ca^{2+}]_i$ in contraction is accomplished by Ca^{2+} entry from extracellular and/or intracellular release of Ca^{2+} from sarcoplasmic reticulum. Between these two Ca^{2+} mobilization pathways, Ca^{2+} influx from extracellular plays a major role in the contraction of colonic smooth muscle^[23–29,34]. L-type voltage-dependent Ca^{2+} channel represents the main route of entry of inward Ca^{2+} current that is gated by potential in smooth muscle cells, particularly in the gastrointestinal tract. Thus, activation of Ca^{2+} channel represents the final ‘common path’ of all mechanisms that regulate muscle contractility^[32,36]. So important are these mechanisms in regulating $[Ca^{2+}]_i$ and the contractile state of smooth muscle that minor defects in function can greatly affect the mechanical activity of colon^[11,30,31,33].

In this study, we investigated the contractile response to KCl and ACh in colonic circular smooth muscle in rats. KCl can induce depolarization of the plasma membrane, activate the voltage-dependent Ca^{2+} channel (dihydropyridine-sensitive L-type Ca^{2+} channel), which results in increase of $[Ca^{2+}]_i$ and

force generation. ACh is one of the most important neurotransmitters in gastrointestinal. ACh can activate Muscarinic M_2 receptors, then non-selective cation channels (NSCC) will be opened. The amount of Ca^{2+} entry through NSCC is controversial, but these channels yield depolarization that activates L-type Ca^{2+} channels, Ca^{2+} entering cells^[25].

We found that the force of contraction in colonic muscle was related nonlinearly to $[Ca^{2+}]_i$; however both velocity and amplitude of $[Ca^{2+}]_i$ increment in muscle cells were directly related to the force generation. Pinaverium bromide is a L-type calcium channel blocker with selectivity for the gastrointestinal used in the treatment of IBS^[37,38,42]. Our work demonstrated that pinaverium inhibited both neurohormonal (ACh) and depolarization (KCl)-induced increment of $[Ca^{2+}]_i$ and contraction of colonic circular muscle in a dose dependent manner. The contractile responses were almost completely blocked at the maximum concentration of 3×10^{-5} mol/L pinaverium, which indicated that repaid influx of Ca^{2+} through L-type calcium channels played a major role in contractions of colon. Pinaverium acted on colonic muscle, and reduced the plateau phase of slow wave, thereby inhibited influx of Ca^{2+} , abolished the accompanying contractile activity^[32]. In normal Ca^{2+} -contain buffer, the contractile responses to ACh and KCl were significantly increased in stress group than those in control group which indicated that more the contractile response was depended on extracellular Ca^{2+} in the stress state and that influx of Ca^{2+} through the cell membrane was increased.

The IC_{50} values (deduced from the concentration-response curves) for inhibition of ACh or KCl induced contraction in the stress group were higher than those in the control group, indicated that increased contractility of stressed colon may in part be due to the increased Ca^{2+} influx through the L-type channels.

Our experiments in insolated circular smooth muscle from rats demonstrated that alterations of the muscle calcium-handling properties was responsible for the increased contractility in colon. However, the precise contribution of these pathways in the intact conscious state can only be speculated. The colonic circular muscle of rat can generate three distinct types of contractions: rhythmic phasic contractions, giant migrating contractions and tone. The amplitudes, durations and motility functions of these contractions differ widely. Because Ca^{2+} is a critical second messenger in the contraction of these cells and the amplitude and duration of cell contraction are correlated with $[Ca^{2+}]_i$, it is likely that the three types of contractions would utilize Ca^{2+} sources differently. The increase of Ca^{2+} influx through L-type channels may be the reason why colonic smooth muscle exhibits hypermotility during stress.

Our study shows that increase of $[Ca^{2+}]_i$ in smooth muscle cells is directly related to the generation of contraction force in colon. L-type Ca^{2+} channels represent the main route of Ca^{2+} entry. Colonic circular muscle hypermotility in rats induced by cold-restraint stress is the result of an increased influx of extracellular Ca^{2+} through L-type Ca^{2+} channels. These studies provide a rationale for the use of pinaverium bromide in the treatment of colonic motor disorders (such as IBS) where excessive contraction need to be suppressed.

REFERENCES

- Janssen HA**, Muris JW, Knotterus JA. The clinical course and prognostic determinants of the irritable bowel syndrom: a literature review. *Scand J Gastroenterol* 1998; **33**: 561-567
- Camilleri M**. Motor function in irritable bowel syndrom. *Can J Gastroenterol* 1999; **13**: 8A-11A
- Ringel Y**, Sperber AD, Drossman DA. Irritable bowel syndrome. *Annu Rev Med* 2001; **52**: 319-338
- Drossman DA**. Review article: an integrated approach to the irritable bowel syndrome. *Aliment Pharmacol Ther* 1999; **13**: 3-14
- Collins SM**. Peripheral mechanisms of symptom generation in irritable bowel syndrome. *Can J Gastroenterol* 2001; **15**: 14B-16B
- Camilleri M**. Review article: clinical evidence to support current therapies of irritable bowel syndrome. *Aliment Pharmacol Ther* 1999; **13**: 48-53
- Camilleri M**. Management of the irritable bowel syndrome. *Gastroenterology* 2001; **120**: 652-668
- Lu CL**, Chen CY, Chang FY, Chang SS, Kang LJ, Lu RH, Lee SD. Effect of a calcium channel blocker and antispasmodic in diarrhoea-predominant irritable bowel syndrome. *J Gastroenterol Hepatol* 2000; **15**: 925-930
- Bouchoucha M**, Faye A, Devroede G, Arzac M. Effect of oral pinaverium bromide on colonic response to food in irritable bowel syndrome patients. *Biomed Pharmacother* 2000; **54**: 381-387
- Scarpignato C**, Pelosini I. Management of irritable bowel syndrome: novel approaches to the pharmacology of gut motility. *Can J Gastroenterol* 1999; **13**: 50A-65A
- Shi XZ**, Sarna SK. Impairment of Ca^{2+} mobilization in circular muscle cells of the inflamed colon. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G234-242
- Mayer EA**, Naliboff BD, Chang L, Coutinho SV. Stress and the Gastrointestinal Tract V. Stress and irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G519-524
- Plourde V**. Stress-induced changes in the gastrointestinal motor system. *Can J Gastroenterol* 1999; **13**: 26A-31A
- Delvaux MM**. Stress and visceral perception. *Can J Gastroenterol* 1999; **13**: 32A-36A
- Bennett EJ**, Tennant CC, Piesse C, Badcock CA, Kellow JE. Level of chronic life stress predicts clinical outcome in irritable bowel syndrome. *Gut* 1998; **43**: 256-261
- Tache Y**, Martinez V, Million M, Rivier J. Corticotropin-releasing factor and the brain-gut motor response to stress. *Can J Gastroenterol* 1999; **13**: 18A-25A
- Drossman DA**, Creed FH, Olden KW, Svedlund J, Toner BB, Whitehead WE. Psychosocial aspects of the functional gastrointestinal disorders. *Gut* 1999; **45**: II25-II30
- Aziz Q**, Thompson DG. Brain-gut axis in health and disease. *Gastroenterology* 1998; **114**: 559-578
- De Schryver AMP**, Samsom M. New developments in the treatment of irritable bowel syndrome. *Scand J Gastroenterol* 2000; **35**: 38-42
- Li JX**, Liu XG, Xie PY, Gu Q, Li J, Zhang Y, Tang CS. The regulation of calcium in the movement of colonic smooth muscle in wrap restraint stress rats. *Zhonghua Neike Zazhi* 2000; **39**: 588-591
- Sanders KM**. Invited review: mechanisms of calcium handling in smooth muscles. *J Appl Physiol* 2001; **91**: 1438-1449
- Bolton TB**, Prestwich SA, Zholos AV, Gordienko DV. Excitation-contraction coupling in gastrointestinal and other smooth muscles. *Annu Rev Physiol* 1999; **61**: 85-115
- Gibson A**, McFadzean I, Wallace P, Wayman CP. Capacitative Ca^{2+} entry and the regulation of smooth muscle tone. *Trends Pharmacol Sci* 1998; **19**: 266-269
- McCarron JG**, Flynn ERM, Bradley KN, Muir TC. Two Ca^{2+} entry pathways mediate $InsP_3$ -sensitive store refilling in guinea-pig colonic smooth muscle. *J Physiol* 2000; **525**(Pt 1): 113-124
- Eglen RM**. Muscarinic receptor and gastrointestinal tract smooth muscle function. *Life Sci* 2001; **68**: 2573-2578
- Bolton TB**, Gordienko DV. Confocal imaging of calcium release events in single smooth muscle cells. *Acta Physiol Scand* 1998; **164**: 567-575
- Young SH**, Ennes HS, McRoberts JA, Chaban VV, Dea SK, Mayer EA. Calcium waves in colonic myocytes produced by mechanical and receptor-mediated stimulation. *Am J Physiol* 1999; **276**: G1204-1212
- Petkov GV**, Boev KK. Control of the phasic and tonic contractions of guinea pig stomach by a ryanodine-sensitive Ca^{2+} store. *Eur J Pharmacol* 1999; **367**: 335-341
- Henkel CC**, Asbun J, Ceballos G, Castillo MC, Castillo EF. Relationship between extra and intracellular sources of calcium and the contractile effect of thiopental in rat aorta. *Can J Physiol Pharmacol* 2001; **79**: 407-414
- Mule F**, Serio R. Increased calcium influx is responsible for the sustained mechanical tone in colon from dystrophic (mdx) mice. *Gastroenterology* 2001; **120**: 1430-1437

- 31 **Sarna SK.** *In vivo* signal-transduction pathways to stimulate phasic contractions in normal and inflamed ileum. *Am J Physiol* 1998; **274**: G618-625
- 32 **Boyer JC,** Magous R, Christen MO, Balmes JL, Bali JP. Contraction of human colonic circular smooth muscle cells is inhibited by the calcium channel blocker pinaverium bromide. *Cell Calcium* 2001; **29**: 429-438
- 33 **Khan I.** Molecular basis of altered contractility in experimental colitis: expression of L-type calcium channel. *Dig Dis Sci* 1999; **44**: 1525-1530
- 34 **Niu WX,** Qin XY, Lu YQ, Shi NC, Wang CP. Role of intracellular calcium in contraction of internal anal sphincter. *World J Gastroenterol* 1999; **5**: 183-184
- 35 **Leahy A,** Epstein O. Non-pharmacological treatments in the irritable bowel syndrome. *World J Gastroenterol* 2001; **7**: 313-316
- 36 **Morel N,** Buryi V, Feron O, Gomez JP, Christen MO, Godfraind T. The action of calcium channel blockers on recombinant L-type calcium channel alpha1-subunits. *Br J Pharmacol* 1998; **125**: 1005-1012
- 37 **Jayanthi V,** Malathi S, Ramathilakam B, Dinakaran N, Balasubramanian V, Mathew S. Role of pinaverium bromide in south Indian patients with irritable bowel syndrome. *J Assoc Physicians India* 1998; **46**: 369-371
- 38 **Poynard T,** Regimbeau C, Benhamou Y. Meta-analysis of smooth muscle relaxants in the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther* 2001; **15**: 355-361
- 39 **Xie DP,** Li W, Qu SY, Zheng TZ, Yang YL, Ding YH, Wei YL, Chen LB. Effect of areca on contraction of colonic muscle strips in rats. *World J Gastroenterol* 2002; **8**: 350-352
- 40 **Li W,** Zheng TZ, Qu SY. Effect of cholecystokinin and secretin on contractile activity of isolated gastric muscle strips in guinea pigs. *World J Gastroenterol* 2000; **6**: 93-95
- 41 **Zheng TZ,** Li W, Qu SY, Ma YM, Ding YH, Wei YL. Effects of Dangshen on isolated gastric muscle strips in rats. *World J Gastroenterol* 1998; **4**: 354-356
- 42 **Villanueva A,** Dominguez-Munoz JE, Mearin F. Update in the therapeutic management of irritable bowel syndrome. *Dig Dis* 2001; **19**: 244-250
- 43 **Cole SJ,** Duncan HD, Claydon AH, Austin D, Bowling TE, Silk DB. Distal colonic motor activity in four subgroups of patients with irritable bowel syndrome. *Dig Dis Sci* 2002; **47**: 345-355
- 44 **Wang XJ,** Wei JG, Wang CM, Wang YC, Wu QZ, Xu JK, Yang XX. Effect of cholesterol liposomes on calcium mobilization in muscle cells from the rabbit sphincter of Oddi. *World J Gastroenterol* 2002; **8**: 144-149

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