Anti-spasmodic effect of Bu-Pi-Yi-Chang pill on colonic contraction of rats

Yue-Qi Wang[1,2], Zheng-Fang Wang[2]*, Sheng-Sheng Zhang[2]*

[1]Beijing University of Chinese Medicine, China
[2]Digestive Disease Center of Beijing Hospital of Traditional Chinese Medicine Affiliated to Capital Medical University, Beijing, China

Abstract

Aim: To investigate the pharmacological effect of Bu-Pi-Yi-Chang (BPYX) pill on colonic contraction of rats and explore its underlying mechanism.

Methods: The experiments were performed on rat isolated colonic longitudinal smooth muscle strips (CLSMS) and were conducted under isometric conditions. CLSMS were suspended in tissue chambers. After stimulation of KCl (80 mM) and acetylcholine (Ach, 0.1 mM), and exhausting intracellular Ca^{2+} and internal flow of extracellular Ca^{2+} to induce muscle contraction, CLSMSs' responses to administration of different dose of BPYX pill were observed. Then on this basis, incubation with different inhibitors was given to verify the mechanism.

Results: BPYC pill dose-dependently and reversibly inhibited colonic contraction. The antispasmodic effect of BPYC pill was partially blocked by 3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester hydrochloride (TMB-8, an intracellular Ca^{2+} antagonist, 500 µM), thapsigargin (a non-competitive inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase, 1 µM), and nifedipine (a voltage-dependent Ca^{2+} channel blocker, 10 µM) ($P<0.05$). However, there were no significant differences after incubation with ethylene glycol tetraacetic acid (EGTA, a calcium chelating agent, 1 mM), 4-aminopyridine (4-AP, a selective blocker of voltage-activated K^+ channel), NG-nitro-L-arginine methyl ester (L-NAME, an inhibitor of nitric oxide synthase, 10 mM), methylene blue (an inhibitor of cyclic guanosine monophosphate, 10 µM), apamin (a selective blocker of Ca^{2+}-activated K^+ channel, 0.1 µM) ($P>0.05$).

Conclusion: BPYC pills could inhibit colonic contraction of rats *in vitro* and its antispasmodic effect is predominantly due to blockade of the calcium channels

Keywords: Muscle contraction, Calcium channels, Herbal medicine, Smooth muscle, Colon

Accepted on May 26, 2017

Introduction

Diarrhea-predominant irritable bowel syndrome (D-IBS) is a chronic gastrointestinal (GI) disease characterized by abdominal pain, discomfort, and changes in bowel movement patterns without any evidence of underlying bowel damage, all of which impair life of patients who burden high healthcare cost [1-3]. The pathogenesis of is not yet fully understood, Because of the complex causes of the D-IBS there is no accepted and effective standard therapy but treatment mostly follows a symptoms oriented trial and error method. Safe and effective treatment options are urgently needed. Some herbal medicines such as the BPYC pill from TCM is traditionally used to treat D-IBS and it can significantly ameliorate abdominal pain, diarrhea and improve patients' quality of life.

It is generally perceived that altered GI motility is mentioned as one of the mechanisms of IBS [4]

Excessive contraction of the intestinal smooth muscle may lead to D-IBS symptoms [5], such as diarrhea and abdominal pain. However, there are no data of BPYC pill and its effect on colonic contractility. GI motility results from coordinated contractions of smooth muscle [6]. Our previous researches found some traditional Chinese medicine and their compounds took effect on regulation of GI motility via inhibiting GI smooth muscle contraction [7,8]. Therefore, the aim of the study at hand was to evaluate the impact of BPYC pill on the motoric activity of CLSMS and investigate the mechanism of its anti-spasmodic effect.

Materials and Methods

Animals

Forty male *Sprague-Dawley* rats (200 ± 20 g) were obtained from Vital River Laboratories Animal Technology Co. Ltd. (Beijing, China) and housed at the animal laboratory of Beijing Chinese Medicine Hospital affiliated to Capital Medical University under a 12 h light/dark cycle (lights on at 08:00 A.M.) with access to food and water *ad libitum*. After acclimatization for three days, rats were fasted for 24 h prior to the experimental treatment.

Drugs and reagents

BPYC pills (provided by Guangzhou Baiyun Mountain Chenliji Pharmaceutical Factory Company, Ltd, Guangzhou, China) are composed of Huangqi (*Radix astragali*), Dangshen (*Radix codonopsis*), Sharen (*Fructus amomi*), Baishao (*Radix paeoniae alba*), Danggui (*Radix angelicae sinensis),* Baizhu

(Rhizoma atractylodis macrocephalae), Rougui (*Cortex cinnamomi*), Yanhusuo (*Rhizoma corydalis*), Lizhihe *(Semen litchi),* Ganjiang (*Rhizoma zingiberis*), Zhigancao (*Radix glycyrrhizae praeparata*), Fangfeng *(Radix saposhnikoviae*), Muxiang (*Radix aucklandiae*), Buguzhi (*Fructus psoraleae*), and Chishizhi (*Halloysitum rubrum*). A decoction of the BPYC pills was prepared with water, concentrated, and freeze-dried into a powder that was stored in sealed bags at 4°C. Before each experiment, BPYC suspension was prepared by mixing the freeze-dried BPYC powder into Krebs solution (KS) to produce different concentrations of BPYC solution.

The following reagents were obtained from Sigma-Aldrich Co. LLC. USA: EGTA (batch number: E0396), Thapsigargin (batch number: T9033), Acetylcholine chloride, (batch number: A6625), Nifedipine (batch number: N7634), TMB-8 (batch number: T111), L-NAME (batch number: N5751), methylene blue (batch number: M9140, 4-AP (batch number: 275875, Apamin (batch nuber: A94590, Nifedipine (batch number:N7634), TMB-8 (batch number: T111), Krebs solution (mM): NaCl 120.6, KCl 5.9, $NaH_2PO_4$1.2, $MgCl^2$ 1.2, $NaHCO_3$ 15.4, $CaCl_2$ 2.5 and glucose 11.5, pH: 7.35-7.45.

Apparatus

A CH-1015 super thermostatic bath (Shanghai Yueping Scientific Instrument Co. Ltd., China) was used for maintaining temperature of 37°C, ML110 Powerlab amplifier, ML740 four-channel recorder and MLT02021D tonotransducer were used for collecting data of tissue tension, and Power Lab/4sp analysis system Labchart7 was used for analysis of physiological data (AD Instruments Shanghai Trading Co. Ltd, Pudong New Area, Shanghai, China).

Tissue preparation

Rats were anesthetized by administering 7% chloral hydrate (35 mg/100 g body weight) via intraperitoneal injections. The distal colon (6–7 cm from the anus) was removed and quickly incised longitudinally along the mesenteric border. Two longitudinal smooth muscle strips (0.8 cm × 0.2 cm) were obtained with the layers of mucosa and sub-mucosa removed [8]. Each strip was suspended in a tissue chamber containing 15 ml Krebs solution that was constantly warmed by circulating water maintained at 37°C and oxygenated with 95% O_2 and 5% CO_2. Portions of CLSMS were ligatured with a medical thread at both ends. One end was fixed to the bottom of the bath (tissue chamber), while the other end was connected to a physiological recorder through the external isometric force transducer (MLT02021D). The initial load of CLSMS samples was 1 g (2 mV) to maintain basal tension. Krebs solution (15 mL, 37°C) was injected into the four water baths that bubbled continuously with 95% O_2 and 5% CO_2. The tissues were allowed to equilibrate for 60 min with rinsing every 15 min before starting the experiment.

Experimental protocol

Effect of the BPYC pill on CLSMS contraction induced by Ach: After equilibration for 60 min, 0.1 mM Ach was added into the Kreb's solution within the bath to induce CLSMS contraction. When regular and stable contractions were obtained, BPYC suspension was added continuously at intervals of 5 min, so that the cumulative concentration of BPYC (Krebs solution as a control) in each chamber reached 3 μg/ml, 10 μg/ml, 30 μg/ml, 100 μg/ml, 300 μg/ml, 1000 μg/ml, 3000 μg/ml, and 10000 μg/ml (crude drug dose). The effect of the BPYC powder on CLSMS contraction was measured as Tension (%) and a dose-dependent curve was obtained from the various BPYC concentrations used, Tension (%)=(tension after agent administration-basal tension)/(tension before agent administration-basal tension) × 100%

Effect of the BPYC pill on CLSMS contraction induced by high K^+: After equilibration for 60 min, 0.1 M KCl was added. Using the same method described above, the effect of the BPYC pill on CLSMS contraction induced by high K^+ was observed through Tension (%) and its associated dose-dependent curve was obtained.

Effect of the BPYC pill on CLSMS contraction, induced by exhausting intracellular Ca^{2+}, and internal flow of extracellular Ca^{2+}: After equilibration of CLSMS for 60 min, normal Krebs solution was replaced with Krebs solution without Ca^{2+}, and 1 μM thapsigargin and 1 mM EGTA were added and incubated for 30 min. Krebs solution without Ca^{2+} was then replaced with Krebs solution containing 2.5 mM Ca^{2+} to induce CLSMS contraction. Using the same methods stated previously, the inhibitory effect of BPYC pill on CLSMS contraction in response to the exhaustion of intracellular Ca^{2+} and subsequent internal flow of extracellular calcium were observed.

Study on the mechanism of effect of the BPYC pill on CLSMS contraction induced by Ach: Using the methods stated above, a chart was prepared to visualize inhibitors' influence on the dose-dependent effect of BPYC. The CLSMS were washed with Krebs solution and different inhibitors (EGTA 1 mM, thapsigargin 1 μM, nifedipine 10 μM, TMB-8 500 μM, methylene blue 10 μM, 4-AP 50 μM, L-NAME 10 mM, apamin 0.1 μM) were added and incubated for 30 min. Following this, 0.1 mM Ach was added to induce CLSMS contraction, and the effect of inhibitors was determined by calculating Inhibition maximum (I_{max}%), half-inhibitory concentration (IC50), and PD value (log IC50). To ensure the experimental effect of individual inhibitor, each strip from eight rats was tested for 3.5–4 h, and only one inhibitor was added to each strip. Group with no incubation of inhibitor is control group. I_{max} (%)=1-(tension after 10000 μg/ml agent - basal tension)/(maximum tension after Ach - basal tension) × 100%.

At the end of each experiment CLSMS were flushed with fresh KS and re-exposed to the reference substance which induces muscle contraction, in order to verify their reactivity.

Animal care and experimental procedures were conducted according to the institutional ethical guidelines and conformed to the requirements of the Institutional Animal Care and Use Committee of Beijing University of Chinese Medicine and approval by Animal Ethics Committee of Beijing Institute of Traditional Chinese Medicine (No.20160601).

Statistical analysis

Data are presented as means ± standard deviation (SD) and n refers to the number of rats. Significance of differences between groups was analyzed using SPSS 20.0 software (SPSS, Chicago, IL, USA) with student *t*-test. $P<0.05$ was considered statistically significant.

Results

Inhibitory effect of the BPYC pill on CLSMS contraction induced by Ach

The addition of 0.1 mM Ach caused muscle tension to increase rapidly to a peak and then decline to plateau at a relatively stable level. The tension reduced in all BPYC and control groups. When BPYC concentration reached 100 μg/ml, there was a significant difference in muscle tension compared to the control group (*$P<0.01$). With increasing concentration, more significant differences were found (**$P<0.001$). At a BPYC concentration of 10,000 μg/ml, tension initially stimulated by Ach decreased by 93% (Figure 1).

(A) Original trace of spontaneous contraction of CLSMS induced by Ach in response to BPYC. Ach: 0.1 mM Ach was added to induce CLSMS contraction.

(B) The concentration-dependently inhibitory effect of the BPYC pill on the tendion of CLSMS induced by Ach. Tension (%)=(tension after agent administration-basal tension)/(tension before agent administration-basal tension) × 100%. The solid line represents the tension (%) of CLSMS in BPYC solution. The dotted line represents the tension (%) of CLSMS in KS. Data were analyzed by student *t*-test and represented as mean ± SD (n=6, *$P=0.0005$, $p<0.01$, **$P<0.001$).

Inhibitory effect of the BPYC pill on CLSMS contraction induced by high K^+

The addition of 0.1 M KCl increased tension to a relatively stable level. After BPYC was added to this solution, a dose-dependent decrease in tension was observed. No change was observed in the control group. When BPYC reached concentrations above 100 μg/ml, a significant difference in muscle tension was measured (**$P<0.01$). At a BPYC concentration of 10,000 μg/ml, tension originally stimulated by high K^+ decreased to 7% of that before the BPYC was added (Figure 2).

(A) Original trace of spontaneous contraction of CLSMS induced by high K^+ in response to BPYC. KCl: 0.1 M KCl was added to induce CLSMS contraction.

(B) The concentration-denpendently inhibitory effect of the BPYC pill on the tension of CLSMS induced by high K^+. Tension (%)=(tension after agent administration-basal tension)/(tension before agent administration-basal tension) × 100%The solid line represents the tension (%) of CLSMS in BPYC solution. The dotted line represents the tension (%) of CLSMS in Krebs solution. Data were analyzed by student *t*-test and represented as mean ± SD (n=4, **$P<0.001$**).**

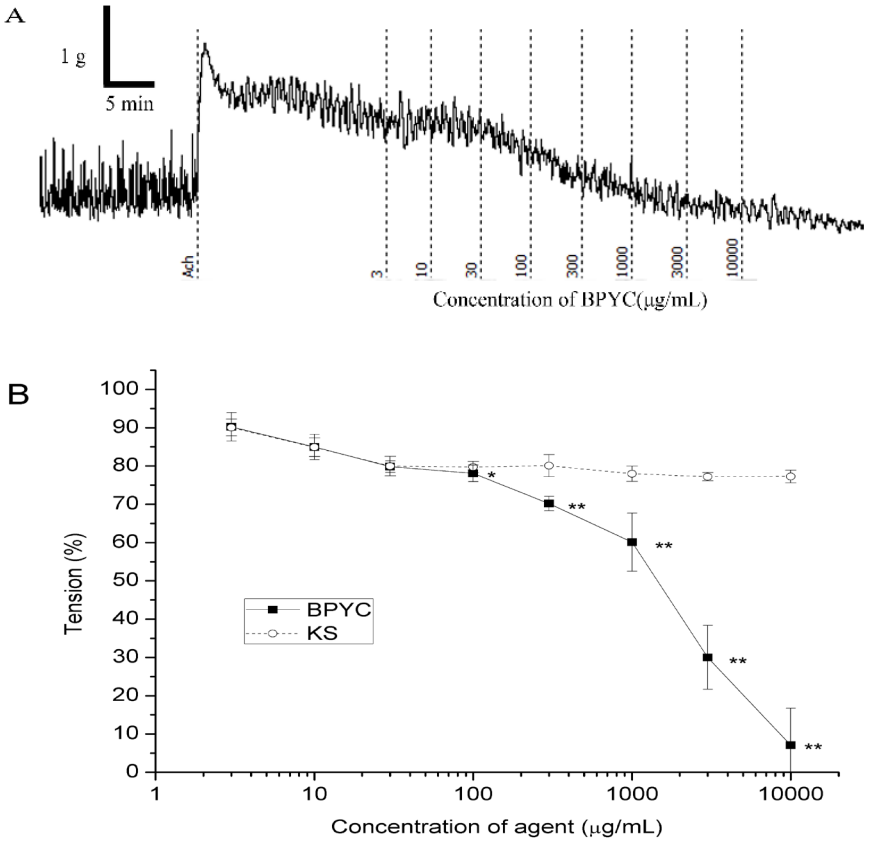

Figure 1. *The inhibitory effect of the BPYC pill on CLSMS contraction induced by Ach.*

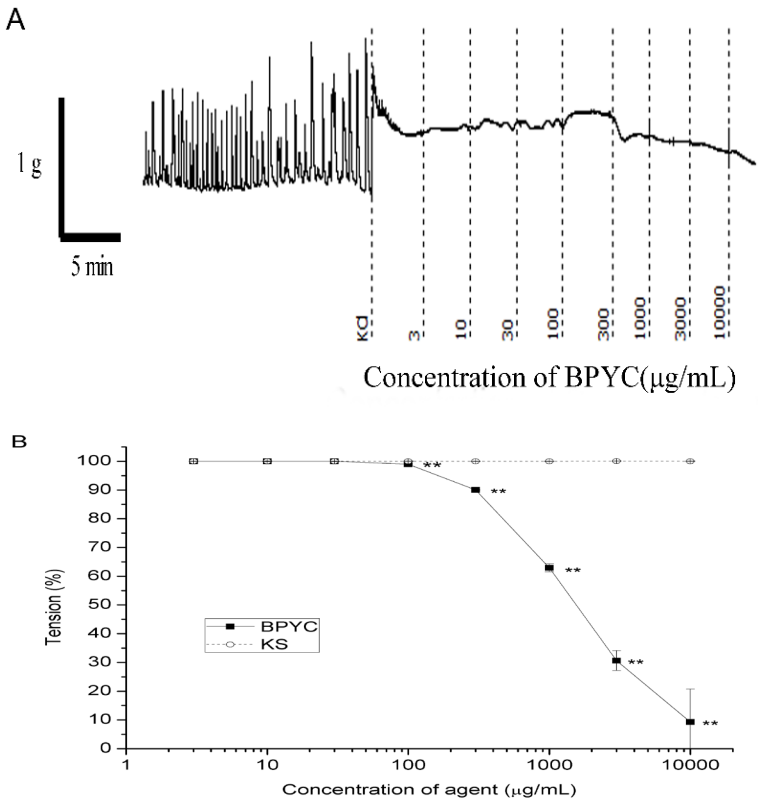

Figure 2. *The inhibitory effect of the BPYC pill on CLSMS contraction induced by high K^+.*

Inhibitory effect of the BPYC pill on CLSMS contraction induced by exhausting intracellular Ca^{2+} and internal flow of extracellular Ca^{2+}

After BPYC was added to CLSMS, muscle tension decreased rapidly while this decrease was much slower in the control group. When the BPYC concentration was more than 10 μg/ml, there was a significant difference compared with the control group (***P*<0.001). At a BPYC concentration of 10,000 μg/ml, tension stimulated by exhausting intracellular Ca^{2+} and internal flow of extracellular calcium decreased to 9% of that before BPYC was added (Figure 3).

(A) Original trace of spontaneous contraction of CLSMS induced by exhausting intracellular Ca^{2+} and resuming the internal flow of extracellular Ca^{2+} in response to BPYC. Normal KS: CLSMS contraction in normal Krebs solution, then replace it with krebs solution without calcium, and incubated with 1 μM thapsigargin and 1 mM EGTA to exhaust intracellular Ca^{2+}, then wash CLSMS with Krebs solution with normal calcium ($CaCl_2$: containing 2.5 mM Ca^{2+}) to induce influx of extracellular Ca^{2+} and CLSMS contraction.

(B) The concentration-denpendently inhibitory effect of the BPYC pill on the tension of CLSMS induced by exhausting intracellular Ca^{2+} and resuming the internal flow of extracellular Ca^{2+}. Tension (%)=(tension after agent administration-basal tension)/(tension before agent administration-basal tension)× 100%. The solid line represents the tension (%) of CLSMS in BPYC solution. The dotted line represents the tension (%) of CLSMS in Krebs solution. Data were analyzed by student *t*-test and represented as mean ± SD (n=4, ***P*<0.001).

Study on the mechanism of inhibitory effect of the BPYC pill on CLSMS contraction induced by Ach

The participation of various ion channels and intercellular pathways were verified by application of several pharmacological inhibitors. After incubation of CLSMS with different inhibitors, I_{max}, IC50, and PD values were assessed. Compared to the control group, there were significant differences between the nifedipine group, the TMB-8 group, and the thapsigargin group (*P*<0.05). However, the EGTA group, L-NAME group, Methylene blue group, 4-AP group, Apamin group showed no significant difference from the control (*P*>0.05) (Table 1).

Notes: I_{max}: Inhibition maximum, I_{max} (%)=1- (tension after 10000 μg/ml agent - basal tension)/(maximum tension after Ach - basal tension) × 100%, IC50: half-inhibitory concentration, PD value=log (IC50). Data were analyzed by student *t*-test and represented as mean ± SD, n=8; ^aP=0.046, ^bP=0.012, ^cP=0.025, ^dP=0.042, ^eP=0.014, ^fP=0.028, ^gP=0.031, ^hP=0.037, ^iP=0.026, *vs* control group.

All reactions caused by BPYC were reversible after flushing with fresh KS.

Discussion

The BPYC pill is an effective Zheng-based drug for D-IBS

Zheng is a key concept of TCM, an ancient medical practice system which emphasizes regulating the integrity of the human body and its interrelationship with natural environments [9]. *Zheng* (meaning syndrome or pattern) is the overall physiological and/or pathological pattern of the human body in response to a given internal and external condition, which usually is

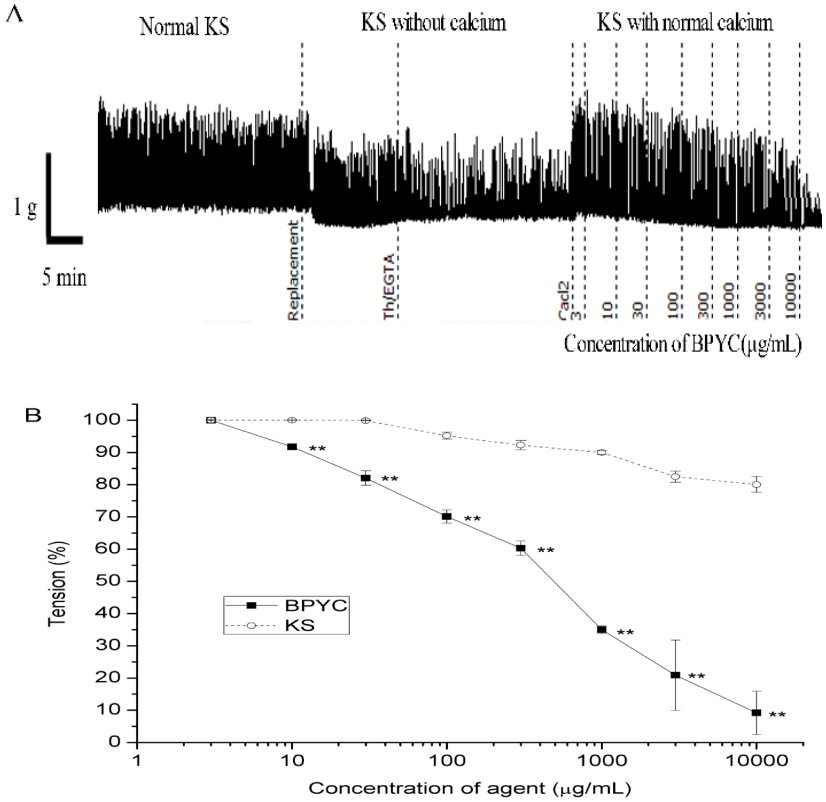

Figure 3. *The inhibitory effect of the BPYC pill on CLSMS contraction induced by exhausting intracellular Ca^{2+} and resuming the internal flow of extracellular Ca^{2+}.*

Table 1*: Influence of different inhibitors on the inhibitory effect of the BPYC pill on CLSMS contraction induced by Ach.*

Group	I_{max}(%)	IC50(ug/ml)	PD-value
Control	99.93 ± 0.19	730.83 ± 98.05	2.65 ± 1.89
EGTA	98.99 ± 0.05	874.55 ± 40.09	2.98 ± 1.78
L-NAME	99.87 ± 0.20	654.32 ± 20.67	2.98 ± 1.46
Methylene blue	99.82 ± 0.06	865.76 ± 41.87	2.56 ± 1.42
4-AP	98.60 ± 2.00	699.08 ± 40.69	2.76 ± 1.43
Apamin	98.77 ± 0.12	712.55 ± 47.39	2.98 ± 2.27
Nifedipine	72.66 ± 2.87[a]	6220.12 ± 59.89[b]	3.20 ± 1.15[c]
TMB-8	68.21 ± 14.33[d]	3701.00 ± 95.03[e]	3.98 ± 1.99[f]
Thapsigargin	79.00 ± 3.86[g]	6587.01 ± 69.98[h]	3.99 ± 1.98[i]

an abstraction of internal disharmony [10]. D-IBS belongs to disease of "Futong (abdominal pain)" and "Xiexie (diarrhea)" with a TCM *Zheng* of Pi-defiency. Bu Pi Yi Chang in Chinese means invigorating Pi and regulating the bowel which is the strategy to treat *Zhen*.. As an important role in BPYC formula, Baizhu were fould to have an analgesic effect on visceral pain and inhibitory effect on bowel movement [tongxie]. Further investigations are needed not only to find effective compounds in BPYC pill, but alse to systematically understand how this recipe can adjust gastrointestinal digestive functions.

BPYC pill could regulate the motility of colonic smooth muscle and its antispasmodic effect is partially mediated by calcium channels

Smooth muscle contraction is controlled by Ca^{2+} signaling [11]. An increase in intracellular Ca^{2+} is the primary trigger of GI smooth muscle contraction [12]. It is confirmed that Ca^{2+} plays a core role in mediating colon smooth muscle contraction [13]. Intracellular free Ca^{2+} levels determine the contraction of smooth muscle cells, and the two main sources of free Ca^{2+} are: (1) the internal flow of extracellular Ca^{2+} and (2) Ca^{2+} release from intracellular storage compartments within the muscle. Among the two, the internal flow of extracellular Ca^{2+} plays the major role [5]. There are three main calcium channels, voltage-operated channels (VOC), receptor-operated channels (ROC), and store-operated channels (SOC). However, in GI smooth muscle, the influx of extracellular Ca^{2+} is achieved mainly through L-type VOC [14,15].

Ach, the most classic and primary excitatory neurotransmitter in the gastrointestinal tract, acts on cell-membrane receptor M to increase intracellular Ca^{2+} concentration, excites gastrointestinal smooth muscle and generates spontaneous contractions, and therefore plays an important role in gastrointestinal movements. Intestinal smooth muscle excitation-contraction coupling caused by Ach leads to an increase in the number of defecations that may lead to diarrhea and other associated symptoms. Smooth muscle contraction, induced by Ach, is involved in both extracellular calcium influx and intracellular calcium storage release. The former is related mainly to VOC and ROC [16]. The latter is involved mainly in G-protein coupling, first by activating protein kinase C (PKC), producing inositol triphosphate (IP_3) that docks to the IP_3 receptor in sarcoplasmic reticulum, opening calcium pools thereby releasing intracellular Ca^{2+} and increasing its concentration. These experimental

results show that the BPYC pill had a concentration-dependently inhibitory effect on CLSMS contraction induced by Ach, which restricts both the internal flow of extracellular calcium and the release of intracellular calcium storage. The possible inhibitory mechanism of internal flow of extracellular calcium may rely on blocking VOC and ROC simultaneously.

It is well known that extracellular high K^+ depolarizes the smooth muscle cell membrane and opens VOC, resulting in an influx of extracellular $Ca^{2+}$and an activation of contractile machinery [17]. This experiment found that BPYC pills inhibit the CLSMS contraction caused by KCl, and its mechanism may be via the inhibition of the internal flow of extracellular Ca^{2+} by blocking VOC.

The antispasmodic effect of BPYC pill was partially blocked by TMB-8, thapsigargin, and nifedipine (P<0.05). Nifedipine has the function of blocking L-VOC [18] and inhibiting the influx of extracellular calcium. In this experiment, after CLSMS incubation with the inhibitor nifedipine for 20 min, Ach was added to stimulate contraction, which was significantly lower than the control group. After administration of different doses of BPYC pill to CLSMS, there was a concentration-dependent inhibitory effect on muscle contraction, reducing muscle tension, and leading to muscle relaxation. Therefore, the BPYC pill may inhibit muscle contraction by blocking VOC and reducing intracellular Ca^{2+} concentration. TMB-8 is capable of inhibiting intracellular calcium release from the sarcoplasmic reticulum, leading to a decline in intracellular calcium concentration. In this experiment, after incubation of CLSMS with the inhibitor TMB-8 for 20 min, Ach was added to stimulate contraction. Contractions of CLSMS in TMB-8 were significantly lower than those of the control group. After administration of different doses of the BPYC pill, there was a concentration-dependent inhibitory effect on muscle contraction leading to reduced muscle tension. Therefore, the BPYC pill may inhibit muscle contraction by promoting reuptake of calcium and inhibiting intracellular calcium release from the sarcoplasmic reticulum. Thapsigargin, as the specific inhibitor of the calcium pump and storage pool, has no inhibitory effect on the calcium pump on the plasma membrane. However, it has been widely used as an agonist of SOC, which inhibits Ca^{2+}-ATPase [19], blocks the reuptake of free Ca^{2+} by the intracellular calcium pool, and increases the Ca^{2+} concentration of cytoplasm rapidly. EGTA, as a Ca^{2+} chelating agent, is capable of chelating extracellular Ca^{2+}. Therefore, using both thapsigargin and EDTA can deplete intracellular Ca^{2+}, which opens SOCs to their maximum capacity. Results of this experiment showed that the BPYC pill had a concentration-dependent inhibitory effect on CLSMS contraction caused by exhausting intracellular Ca2+ and internal flow of extracellular calcium, and it is speculated that the BPYC pill can block SOC.

However, it must be emphasized that the obtained results are not suffcient to assign BPYC pill as an agonist or an antagonist of VOC, ROC or SOC. Nevertheless, VOC, ROC and SOC certainly participate in BPYC pill mediated effect. To verify its mechanism of reducing intracellular calcium or inhibiting calcium channels, further studies should use the technique of calcium imaging or whole-cell patch clamp. In addition, GI motility is mainly modulated by enteric nervous system (ENS), intestine cell of cajal (ICC) and smooth muscle. It cannot be excludes the possibility that the inhibitory effect of BPYC on CLSMS is via the ENS and ICC. Since none of the evaluated inhibitory pathways explains fully the mechanism of BPYC pill's antispasmodic effect, further mechanistic investigation is required. Considering the effect of other TCM formula on gut smooth muscle [20], further exploration of calcium-mediated mechanisms should be performed as a continuation of the present study.

Conclusion

BPYC pills could inhibit colonic contraction of rats *in vitro* and its antispasmodic effect is partially mediated by calcium channels, possibly by blocking the internal flow of extracellular Ca^{2+}, inhibiting the release of intracellular Ca^{2+}, and reducing free intracellular Ca^{2+} concentration via VOC, ROC, and SOC, respectively.

Acknowledgement

This study was supported by grant from the Clinical Medicine Development Project of Beijing Municipal Administration of Hospitals (No. ZYLX201411).

Conflict of Interest

All authors declare no conflict of interest in association with this work.

References

1. Longstreth GF, Thompson WG, Chey WD, et al. Functional bowel disorders. Gastroenterol. 2009;130(5):1480–491.
2. Yacavone RF, Locke GRI, Provenzale DT, et al. Quality of life measurement in gastroenterology: what is available? Am J Gastroenterol. 2001;96(2):285–97.
3. Sandler RS, Everhart JE, Donowitz M, et al. The burden of selected digestive diseases in the United States. Gastroenterol. 2002;122(5):1500–511.
4. Canavan C, West J, Card T. The epidemiology of irritable bowel syndrome. Clin Epidemiol. 2014;6:71–80.
5. Dai Y, Liu JX, Li JX, et al. Effect of pinaverium bromide on stress-induced colonic smooth muscle contractility disorder in rats. World J Gastroenterol. 2003;9(3):557-61.
6. Sanders KM, Koh SD, Ro S, et al. Regulation of gastrointestinal motility—Insights from smooth muscle biology. Nat Rev Gastroenterol Hepatol. 2012;9(11):633-45.
7. Wu ZY, Zhang SS, Li PC, et al. Effect of *Aurantii fructus immaturus* flavonoid on the contraction of isolated gastric smooth muscle strips in rats. Evidence Based Complement Alternat Med. 2016.
8. Yang C, Zhang SS, Li XL, et al. Inhibitory effect of Tong Xie-Yao Fang formula on colonic contraction in rats. World J Gastroenterol. 2015;21(10):2912–917.
9. Federoff HJ, Gostin LO. Evolving from reductionism to holism: is there a future for systems medicine? JAMA. 2009;302(9):994–96.

10. Wang, YH, Xu D, et al. A systems biology approach to diagnosis and treatments. Science. 2014;346:S13-S15.

11. Berridge MJ. Smooth muscle cell calcium activation mechanisms. J Physiol. 2008,586(21):5047-061.

12. Perrino BA. Calcium sensitization mechanisms in gastrointestinal smooth muscles. J Neurogastroenterol Motil. 2016;22(2):213-25.

13. Kinoshita K, Sato K, Hori M, et al. Decrease in activity of smooth muscle L-type Ca^{2+} channels and its reversal by NF-kappa-B inhibitors in Crohn's colitis model. Am J Physiol Gastrointest Liver Physiol. 2003;285(3):G483-493.

14. McCarron JG, Craig JW, Bradley KN, et al. Agonist-induced phasic and tonic responses in smooth muscle are mediated by InsP(3). J Cell Sci. 2002;115(10):2207-218.

15. Zhang WW, Li Y, Wang XQ, et al. Effects of magnolol and honokiol derived from traditional Chinese herbal remedies on gastrointestinal movement. World J Gastroenterol. 2005;11(28): 4414-418.

16. Zhang M, Leung FP, Huang Y, et al. Increased colonic motility in a rat model of irritable bowel syndrome is associated with up-regulation of L-type calcium channels in colonic smooth muscle cells. Neurogastroenterol Motil. 2010;22(5):162-70.

17. Chou TC. New mechanisms of antiplatelet activity of nifedipine, an L-type calcium channel blocker. Biomedicine. (Taipei) 2014;4(4):24.

18. Rogers TB, Inesi G, Wade R, et al. Use of thapsigargin to study Ca^{2+} homeostasis in cardiac cells. Biosci Rep. 1995;15(5):341-49.

19. Chen G, Zhu L, Liu Y, et al. Isoliquiritigenin, a flavonoid from licorice, plays a dual role in regulating gastrointestinal motility *in vitro* and *in vivo*. Phytother. Res. 2009;23(4):498–06.

20. Mendel M, Chłopecka M, Dziekan N, et al. Antispasmodic effect of selected Citrus flavonoids on rat isolated jejunum specimens. Eur J Pharmacol. 2016;791:640-46.

***Correspondence to:**

Professor Zheng-Fang Wang and Sheng-sheng Zhang
Digestive Disease Center of Beijing Chinese Medicine Hospital Affiliated to Capital Medical University
23 Meishuguanhou Street, Dongcheng District
Beijing, 100010, China
E-mail: bjwzf0442@163.com; zhss2000@163.com