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• *BRIEF REPORTS* •

Effect of rhubarb on contractile response of gallbladder smooth muscle strips isolated from guinea pigs

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Abstract

AIM: To investigate the effect of rhubarb on contractile response of isolated gallbladder muscle strips from guinea pigs and its mechanism.

METHODS: Guinea pigs were killed to remove the whole gallbladder. Two or three smooth muscle strips (8 mm×3 mm) were cut along the longitudinal direction. The mucosa on each strip was carefully removed. Each longitudinal muscle strip was suspended in a tissue chamber containing 5 mL Krebs solution (37 ℃), bubbled continuously with 950 mL/L O_2 and 50 mL/L CO_2. The resting tension (g), mean contractile amplitude (mm), and contractile frequency (waves/min) were simultaneously recorded on recorders. After 2-h equilibration, rhubarb (10, 20, 70, 200, 700, 1 000 g/L) was added cumulatively to the tissue chamber in turns every 2 min to observe their effects on gallbladder. Antagonists were given 3 min before administration of rhubarb to investigate the possible mechanism.

RESULTS: Rhubarb increased the resting tension (from 0 to 0.40±0.02, P<0.001), and decreased the mean contractile amplitude (from 5.22±0.71 to 2.73±0.41, P<0.001). It also increased the contractile frequency of the gallbladder muscle strips in guinea pigs (from 4.09±0.46 to 6.08±0.35, P<0.001). The stimulation of rhubarb on the resting tension decreased from 3.98±0.22 to 1.58±0.12 by atropine (P<0.001), from3.98±0.22 to 2.09±0.19 by verapamil (P<0.001) and from 3.98±0.22 to 2.67±0.43 by phentolamine (P<0.005). But the effect was not inhibited by hexamethonium (P>0.05). In addition, the action of mean amplitude and frequency was not inhibited by the above antagonists.

CONCLUSION: Rhubarb can stimulate the motility of isolated gallbladder muscle strips from guinea pigs. The stimulation of rhubarb might be relevant with M receptor, Ca^{2+} channel and α receptor partly.

Key words: Rhubarb; Gallbladder smooth muscle strips; M receptor; Ca^{2+} channel; α receptor

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INTRODUCTION

Muscular contraction of the gallbladder is the primary determinant factor of bile delivery into the duodenum. Rhubarb has been reported to promote bile secretion and dredge liver fine bile ducts. Moreover, rhubarb relaxes Oddi sphincter. It has been used to treat cholecystitis and bile duct infection, which was caused by manifold bacteria. But the action and mechanisms of rhubarb on the gallbladder smooth muscle strips *in vitro* are not reported. In this study , we observed the effects of rhubarb on the gallbladder muscle strips isolated from guinea pigs and investigated the possible mechanism concerned.

MATERIALS AND METHODS

Animal preparation

Guinea pigs of either sex (grade I, purchased from Animal Center of Lanzhou Biology Institute), weighing 350-450 g, were fasted with free access to water for 24 h, and killed to remove the whole gallbladder. Two or three smooth muscle strips (8 mm×3 mm) were cut along the longitudinal direction. The mucosa on each strip was carefully removed.

Experiment

The muscle strips were suspended in a tissue chamber containing 5 mL Krebs solution, constantly warmed by a circulating water jacket at 37 ℃, bubbled continuously with 950 mL/L O_2 and 50 mL/L CO_2. One end of the strip was fixed to a hook on the bottom of the chamber. The other end was connected to an external isometric force transducer (JZ-BK, BK). The preparation was subjected to 1-g-load tension and washed with 5 mL Krebs solution every 20 min. The motility of gallbladder strips in tissue chambers was

simultaneously recorded on ink writing two channel recorders (LMS_ZB, Chengdu). After 2-h equilibration, 10, 20, 70, 200, 700, 1 000 g/L of rhubarb were added cumulatively in turns every 2 min to observe their effects on gallbladder. Cumulating final concentration of rhubarb was 0.05, 0.15, 5, 15, 50, 100 g/L in the tissue chamber. Atropine (1 μmol/L), hexamethonium (10 μmol/L), phentolamine (1 μmol/L) and verapamil (0.05 μmol/L) were added 3 min before the administration of rhubarb to investigate whether the actions of rhubarb were relevant with M receptor, α receptor, Ca^{2+} channel and N receptor. The concentrations of antagonists were the final concentrations[1].

Drug preparation

Rhubarb was broken into pieces, boiled, filtrated, and diluted to 1 000 g/L, and then diluted to 10, 20, 70, 200, 700, 1 000 g/L (the drug was appraised and prepared by Drug Control Institute of Gansu Province). The following antagonists were used: atropine (Pharmaceutical Factory in Yancheng, Jiangsu Province), hexamethonium (Sigma Chemical Company), phentolamine (Beijing Thirteen Pharmaceutical Factory), and verapamil (Lanzhou Pharmaceutical Factory).

Data analysis

The results were presented as mean±SE, and statistically analyzed by ANOVA, $P<0.05$ was considered statistically significant.

RESULTS

Effect of rhubarb on spontaneous contraction of gallbladder muscle strips

Rhubarb (0.05, 0.15, 5, 15, 50, 100 g/L) increased the resting tension, and decreased the mean contractile amplitude. It also increased the contractile frequency of gallbladder muscle strips isolated from guinea pigs (Figure 1).

Effect of atropine, verapamil, phentolamine, and hexamethonium on responses caused by rhubarb

Atropine (1 μmol/L), hexamethonium (10 μmol/L), phentolamine (1 μmol/L) and verapamil (0.05 μmol/L) had no significant effect on gallbladder muscle strips isolated from guinea pigs. But when given 3 min before the administration of rhubarb (0.05, 0.15, 5, 15, 50, 100 g/L), atropine, verapamil, and phentolamine reduced the increasing action of rhubarb on the resting tension of gallbladder muscle strips at different degrees (Table 1). They had no significant effects on the other actions of rhubarb (Tables 2, 3). Hexamethonium given 3 min before the administration of rhubarb had no significant effects on the action of rhubarb.

DISCUSSION

The effects of Chinese herbs on the gallbladder motility have been reported[2-5]. It was reported that rhubarb could be used to treat cholecystitis[6-9] based on the fact that some substances extracted from rhubarb have significant effects on promoting gallbladder contraction. Also, rhubarb can relax Oddi sphincter and restrain the activity of pancreas succus amylase. These effects may be the basis of treating acute pancreas adenitis. But investigation of the effects of rhubarb on gallbladder smooth muscle *in vitro* is rare. In this experiment, we found that rhubarb significantly increased the resting tension and contractile frequency of isolated guinea pig gallbladder strips, and the mean amplitude

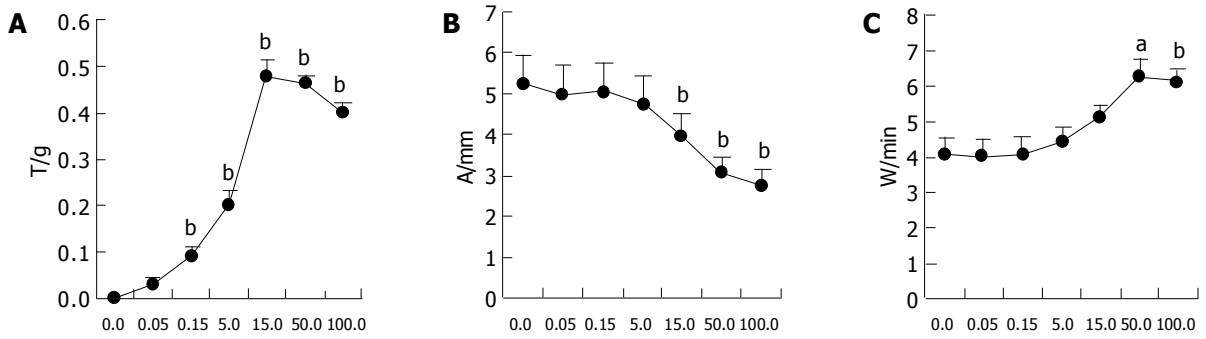

Figure 1 Effect of rhubarb on resting tension (g), mean contractile amplitude (mm) and frequency (waves/min) of isolated guinea pig gallbladder muscle strips (n = 12). A: resting tension; B: the mean contractile amplitude; C: frequency. $^{a}P<0.005$, $^{b}P<0.001$ *vs* control (the gallbladder spontaneous contraction under 1 g initial load when rhubarb was 0 g/L). The resting tension of each strip in control was 0.

Table 1 Effect of rhubarb on resting tension (g) of isolated guinea pig gallbladder muscle strips after pretreatment with antagonists (mean±SE)

Resting tension (g)	Rhubarb (g/L)						
	0	0.05	0.15	5	15	50	100
Rhubarb	0	0.03±0.01	0.09±0.02^{c}	0.20±0.03^{c}	0.48±0.03^{c}	0.46±0.02^{c}	0.40±0.02^{c}
Phe+Rhu	0	0.04±0.01	0.06±0.01^{e}	0.11±0.01^{c,g}	0.33±0.05^{c,g}	0.30±0.05^{c,g}	0.27±0.04^{c,g}
Hex+Rhu	0	0.04±0.01	0.08±0.01^{e}	0.17±0.02^{g}	0.39±0.02^{g}	0.40±0.02^{g}	0.35±0.02^{g}
Atr+Rhu	0	0^{g}	0.03±0.01^{g}	0.07±0.01^{a,g}	0.22±0.04^{c,g}	0.19±0.03^{c,g}	0.16±0.01^{c,g}
Iso+Rhu	0	0.03±0.01	0.07±0.01^{c}	0.10±0.01^{c,e}	0.30±0.01^{c,g}	0.28±0.02^{c,g}	0.21±0.02^{c,g}

$^{a}P<0.05$, $^{c}P<0.005$ *vs* control (the gallbladder spontaneous contraction under 1-g initial load when rhubarb was 0 g/L) n = 12. The resting tension of each strip in control was 0. $^{e}P<0.05$, $^{g}P<0.005$ *vs* rhubarb (the resting tension of adding each concentration of rhubarb) n = 12.

Table 2 Effect of rhubarb on the contractile amplitude (mm) of isolated guinea pig gallbladder muscle strips after pretreatment with antagonists (mean±SE)

Amplitude (mm)	Rhubarb (g/L)						
	0	0.05	0.15	5	15	50	100
Rhubarb	5.2±0.7	4.9±0.7	5.0±0.7	4.8±0.7	4.0±0.5	3.0±0.4[a]	2.7±0.4[c]
Phe+Rhu	3.3±0.4	3.2±0.4	3.1±0.4	3.0±0.4	2.9±0.4	2.3±0.4	1.7±0.4[c]
Hex+Rhu	4.7±0.6	4.5±0.6	4.3±0.6	4.2±0.6	3.8±0.8	3.1±0.5	2.7±0.5[a]
Atr+Rhu	5.4±0.9	5.3±0.9	5.1±0.8	4.8±0.7	4.5±0.7	3.8±0.6	3.1±0.5[a]
Iso+Rhu	5.2±0.9	5.1±1.0	4.9±1.0	4.7±0.8	4.4±0.9	3.4±0.6	3.0±0.5

[a]$P<0.05$, [c]$P<0.005$ *vs* control (the gallbladder spontaneous mean contraction amplitude under 1-g initial load when rhubarb was 0 g/L) $n = 12$.

Table 3 Effect of rhubarb on the contractile frequency (waves/min) of isolated guinea pig gallbladder muscle strip after pretreatment with antagonists (mean±SE)

Frequency (w/min)	Rhubarb (g/L)						
	0	0.05	0.15	5	15	50	100
Rhubarb	4.1±0.5	4.0±0.4	4.1±0.5	4.4±0.4	5.0±0.3	6.3±0.5[c]	6.1±0.4[c]
Phe+rhu	4.2±0.5	4.5±0.5	4.6±0.6	4.6±0.5	4.8±0.6	5.5±0.6	6.0±0.5[c]
Hex+rhu	3.7±0.3	3.8±0.4	3.8±0.4	4.1±0.5	4.9±0.6	5.3±0.7[c]	5.5±0.5[c]
Atr+rhu	3.5±0.4	3.4±0.4	3.5±0.4	3.7±0.4	4.0±0.4	4.8±0.5[c]	5.0±0.5[c]
Iso+rhu	4.1±0.4	4.1±0.4	4.1±0.4	4.2±0.4	4.5±0.4	5.0±0.5	4.9±0.4

[a]$P<0.05$, [c]$P<0.005$ *vs* control (the gallbladder spontaneous contraction frequency under 1-g initial load when rhubarb was 0 g/L) $n = 12$.

decreased at the same time. Atropine, phentolamine and verapamil could block this exciting action partly, whereas hexamethonium had no inhibitory effects. Our results suggested that the stimulating action of rhubarb on gallbladder smooth muscle strips was relevant with M receptor, Ca^{2+} channel and α receptor, but irrelevant with N receptors.

The presence of M receptors in guinea pig gallbladder smooth muscle cells has been reported recently[10-12]. The majority of these receptors are said to be M_2 subtype. However, there are controversial reports about the functional muscarinic receptors that mediate contraction in this tissue. Kurtel *et al*[13] presumed that M_4 receptors and M_5 receptors played a major role in gallbladder contraction. But von Schrenck *et al*.[11] reported that M_3 receptors mediated the movement. The study of Akici *et al*[14] supported the conclusion that the majority of muscarinic receptors of M_2 did not mediate the contractile responses. When M receptors were stimulated, the potential sensitive Ca^{2+} channels were opened, which would cause the influx of extracellular Ca^{2+} and induce the contraction of smooth muscles. The key determinant of smooth muscle contractility is the concentration of intracellular free calcium Ca^{2+}, which could trigger a sequence of events leading to the generation of forces[15-17]. When smooth muscle cells were stimulated, extracellular Ca^{2+} entered into cells. Meanwhile sarcoplasmic reticuli could bring into play the function of Ca^{2+}. When some exciting transmitters, hormones and drugs combined with muscular receptors, the secondary message was generated via G protein, then Ca^{2+} was released. The result was a rise of $[Ca^{2+}]$. This increased calcium sequentially bound to the four binding sites on the regulatory protein, calmodulin (CAM). The activated calmodulin bound to myosin light chain kinase (MLCK) to form an active complex ($Ca^{++}{}_4$CaM.MLCK). Phosphorylation of myosin produced a conformational change in the myosin head group that could activate ATPase. The interaction between the phosphorylated myosin heads and actin filaments generated forces, filament movements and cell shorting, with sequential attachment and detachment of the cross-bridges, leading to contraction[18]. In this experiment, verapamil (an inhibitor of Ca^{2+} channel) significantly blocked the contraction response of rhubarb. This result is consistent with the above results. α receptor has been found in gallbladder smooth muscles that could mediate the exciting action. After phentolamine (an inhibitor of α receptor) was added, the contraction response of rhubarb significantly decreased. This mechanism will allow us to gain more information about the effects of rhubarb on gallbladder.

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