

## Characteristics of deslanoside-induced modulation on jejunal contractility

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### Abstract

**AIM:** To characterize the dual effects of deslanoside on the contractility of jejunal smooth muscle.

**METHODS:** Eight pairs of different low and high contractile states of isolated jejunal smooth muscle fragment (JSMF) were established. Contractile amplitude of JSMF in different low and high contractile states was selected to determine the effects of deslanoside, and Western blotting analysis was performed to measure the effects of deslanoside on myosin phosphorylation of jejunal smooth muscle.

**RESULTS:** Stimulatory effects on the contractility of JSMF were induced ( $45.3\% \pm 4.0\%$  vs  $87.0\% \pm 7.8\%$ ,  $P < 0.01$ ) by deslanoside in 8 low contractile states, and inhibitory effects were induced ( $180.6\% \pm 17.8\%$  vs  $109.9\% \pm 10.8\%$ ,  $P < 0.01$ ) on the contractility of JSMF in 8 high contractile states. The effect of deslanoside on the phosphorylation of myosin light chain of JSMF in low ( $78.1\% \pm 4.1\%$  vs  $96.0\% \pm 8.1\%$ ,  $P < 0.01$ ) and high contractile state ( $139.2\% \pm 8.5\%$  vs  $105.5 \pm 7.34$ ,  $P < 0.01$ ) was also bidirectional. Bidirectional regulation (BR) was abolished in the presence of tetrodotoxin. Deslanoside did not affect jejunal contractility pretreated with the  $\text{Ca}^{2+}$  channel blocker verapamil or in a  $\text{Ca}^{2+}$ -free assay condition. The stimulatory effect of deslanoside on JSMF in a low contractile state (low  $\text{Ca}^{2+}$  induced) was abolished by atropine. The inhibitory effect of deslanoside on jejunal contractility in a high contractile state (high  $\text{Ca}^{2+}$  induced) was blocked by phentolamine, propranolol and L-NG-nitro-arginine, respectively.

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**CONCLUSION:** Deslanoside-induced BR is  $\text{Ca}^{2+}$  dependent and is related to cholinergic and adrenergic systems when JSMF is in low or high contractile states.

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**Key words:** Deslanoside; Bidirectional regulation; Contractile state; Jejunal smooth muscle

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### INTRODUCTION

More than 200 naturally occurring cardiotonic glycosides (CGs, cardiac glycosides) have been identified to date<sup>[1]</sup>.

CGs have long been and will continue to be used in the treatment of congestive heart failure and have entered the clinical trial phase for treating cancer<sup>[2-5]</sup>. CGs enhance the myocardial contraction by increasing intracellular  $\text{Ca}^{2+}$  *via* inhibiting the activities of  $\text{Na}^+/\text{K}^+$ -ATPase<sup>[6-8]</sup>. CGs ouabain has been found to induce excitation on colonic smooth muscle<sup>[9]</sup>. Toxic effects of CGs are observed in clinics, e.g., atrioventricular block, bradycardia, and gastrointestinal irritation<sup>[1]</sup>. Probably due to the fact that no therapeutic applications are yet known, the characteristics of CGs on the intestinal motility have rarely been investigated.

Intestinal motility is mainly modulated by neurotransmitters and hormones; the neuronal regulation of intestinal motility involves intrinsic, e.g., enteric nervous system (ENS), as well as extrinsic nerves, e.g., the sympathetic and parasympathetic nervous system (SPNS)<sup>[10]</sup>. The central nervous system is able to modulate, but not entirely control, the motor activity by sending instructions *via* SPNS, and ENS modulates the motility of intestinal smooth muscle even when isolated from the body to fulfill pivotal functions<sup>[10,11]</sup>. In this study, we proposed a hypothesis that inducible bidirectional regulation (BR) is the major autonomous control of intestinal motility in the absence of CNS control, and that both low and high contractile states of intestinal smooth muscle can be regulated back toward normal contractile state by a single CGs deslanoside-induced BR. To test the hypothesis, different low and high contractile states of intestinal smooth muscle were established. Considering both colon and small intestine are sites of “abnormal” motility in intestinal smooth muscle disorders, e.g., irritable bowel syndrome (IBS)<sup>[12-14]</sup>, and that the jejunum is a “typical” region of the small intestine, we chose to investigate the contractility of isolated jejunal smooth muscle fragment (JSMF) and its underlying mechanisms involved in deslanoside-induced BR.

## MATERIALS AND METHODS

### Experimental models of diarrhea and constipation

The animal protocol was approved by Dalian Medical University Animal Care and Ethics Committee, and all experimental procedures described were carried out in accordance with the Declaration of Helsinki. Sprague-Dawley rats (200-250 g) were used in the assay. Constipation-predominant (CP) rats were established by daily gavage with cool water (0 °C-4 °C) for 14 d, and the control rats were prepared by daily gavage with water at room temperature<sup>[15,16]</sup>. Diarrhea-predominant (DP) rats were established by intracolonic instillation of acetic acid and restraint stress, and control rats received intracolonic instillation with saline<sup>[17-19]</sup>. The granule number and the moisture content of the feces from the control group and the model group were measured daily, and the body mass was recorded once every 3 d.

### Tissue preparation

Tissue fragments from the intact tubular jejunum were

prepared according to the methods described previously<sup>[20,21]</sup>. Jejunum was isolated from normal, CP and DP rats. Jejunal fragments were cut into approximately 2 cm in length (tubes). One end of the jejunal fragment in longitudinal direction was fixed to the wall of a tissue bath chamber (20 mL volume), and the other end was connected to a force-displacement transducer. This montage measured the contractile response of JSMF.

### Contractility determination

The organ bath was maintained at 37 °C, and the resting tension was set optimally at 1.0 g. Preliminary experiments showed that this load stretched tissues to their optimal length for force development during contraction. JSMF was allowed to equilibrate in aerated Krebs buffer for 50 min and the bath solution was replaced every 10 min. Contractile amplitude of JSMF was measured from the baseline to the peak and was expressed as a percentage of normal contractile amplitude. Contractile amplitude was recorded and identical time-interval of each assay with the same start and stop time was chosen to compare the amplitude before and after drug treatment in different assay conditions. The mean amplitude was calculated from six independent assays.

### Ex vivo assay condition

The contractility of JSMF was measured in Krebs buffer (118 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L  $\text{KH}_2\text{PO}_4$ , 1.2 mmol/L  $\text{MgSO}_4$ , 4.2 mmol/L  $\text{NaHCO}_3$ , 2.5 mmol/L  $\text{CaCl}_2$ , 10 mmol/L glucose; pH 7.4) and selected as the normal contractile state (NCS). The jejunal contractility measured in modified low  $\text{Ca}^{2+}$  (1.25 mmol/L) and high  $\text{Ca}^{2+}$  (5.0 mmol/L) Krebs buffer was selected as the representative low contractile state (RLCS) and representative high contractile state (RHCS), respectively, since spontaneous contractions of intestinal smooth muscle were paralleled to intracellular  $\text{Ca}^{2+}$  concentration<sup>[22,23]</sup>. One pair of low-high contractile states was established from jejunal smooth muscle isolated from CP and DP rats. The other six pairs of low-high contractile states were generated by incubating JSMF in modified low  $\text{K}^+$  (2.5 mmol/L)-high  $\text{K}^+$  (10.0 mmol/L) Krebs buffer, low  $\text{Na}^+$  (100 mmol/L)-high  $\text{Na}^+$  (150 mmol/L) Krebs buffer, high  $\text{Mg}^{2+}$  (3.0 mmol/L)-low  $\text{Mg}^{2+}$  (1.0 mmol/L) Krebs buffer, adrenaline (5.0  $\mu\text{mol/L}$ )-ACh (5.0  $\mu\text{mol/L}$ ) Krebs buffer, quercetin (10.0  $\mu\text{mol/L}$ )-capsaicin (10.0  $\mu\text{mol/L}$ ) and nitric oxide (NO) donor sodium nitroprusside (SNP) (5  $\mu\text{mol/L}$ )-erythromycin (10  $\mu\text{mol/L}$ ) Krebs buffer<sup>[24,25]</sup>. After the stable contractile state of jejunal contraction was obtained, deslanoside was added to the bath to make a final concentration of 20  $\mu\text{mol/L}$ , unless otherwise indicated.

### Western blotting analysis

The phosphorylation of myosin light chain (PMLC) in jejunum was examined by Western blotting as described previously<sup>[26,27]</sup>. JSMF was immediately treated with low  $\text{Ca}^{2+}$  or high  $\text{Ca}^{2+}$  Krebs buffer for 1 min in the absence or presence of 20  $\mu\text{mol/L}$  deslanoside, and then were

**Table 1** Effects of deslanoside on the contractility of jejunal smooth muscle pretreated with receptor antagonist

Agents	Normal contractile state		Low contractile state		High contractile state	
	Pre-deslanoside	Post-deslanoside	Pre-deslanoside	Post-deslanoside	Pre-deslanoside	Post-deslanoside
Krebs buffer	100.0 ± 12.1	149.0 ± 13.0 <sup>b</sup>	39.1 ± 2.8	89.1 ± 5.1 <sup>b</sup>	177.7 ± 16.0	109.3 ± 11.9 <sup>b</sup>
Atropin	93.3 ± 6.3	155.0 ± 15.1 <sup>b</sup>	29.1 ± 1.1	32.4 ± 3.3	149.7 ± 11.0	100.3 ± 11.2 <sup>b</sup>
Diphenhydramine	109.0 ± 14.3	145.2 ± 13.1 <sup>b</sup>	46.5 ± 3.8	94.0 ± 4.9 <sup>b</sup>	169.6 ± 15.2	108.5 ± 11.6 <sup>b</sup>
Cimetidine	100.0 ± 11.8	153.4 ± 13.0 <sup>b</sup>	42.9 ± 3.9	93.4 ± 8.1 <sup>b</sup>	180.1 ± 17.4	113.3 ± 12.1 <sup>b</sup>
Phentolamine	92.5 ± 6.5	160.6 ± 16.2 <sup>b</sup>	55.4 ± 5.5	90.6 ± 5.2 <sup>b</sup>	189.3 ± 19.2	184.5 ± 17.2
Propranolol	89.4 ± 9.8	145.5 ± 14.2 <sup>b</sup>	33.2 ± 2.6	95.6 ± 7.8 <sup>b</sup>	163.1 ± 16.1	161.7 ± 17.3
L-NNA	103.0 ± 11.3	150.0 ± 13.2 <sup>b</sup>	51.3 ± 4.6	119.6 ± 12.5 <sup>b</sup>	190.5 ± 18.2	195.1 ± 19.1

Contractile amplitude of isolated jejunal smooth muscle fragment in normal contractile state is defined as 100% (control). Other data are the relative value (%) compared with control, and are expressed as the mean ± SE; <sup>b</sup>*P* < 0.01 *vs* the data prior to deslanoside treatment (*n* = 6). L-NNA: L-NG-nitroarginine.

frozen and stored in liquid nitrogen. Ground product was incubated for 30 min in ice-cold homogenization buffer. The blots on nitrocellulose filter membrane were probed with phosphor-myosin light chain 2 (Ser 19) antibody (1:1000) [No. 3671, Cell Signaling Technology, Inc (CST), United States] and myosin light chain 2 (total myosin light chain) antibody (1:1000) (No. 3672, CST, United States) at 4 °C with gentle shaking overnight. Anti-rabbit IgG secondary antibodies were used at 1:2500 for 60 min at room temperature and bands were detected and quantified using Multispectral imaging system (UVP, United States).

### Drugs

Injectable deslanoside was obtained from Sine Pharmaceutical (Shanghai, China). Capsaicin and quercetin was purchased from Chengdu Biopurify Phytochemicals Co. Ltd, China. Tetrodotoxin (TTX) was a product of Aladdin Chemistry Co. Ltd (Shanghai, China). Unless otherwise indicated, chemicals were obtained from Sigma (United States).

### Statistical analysis

Student's *t* test was used to compare statistical differences between two groups, and *P* < 0.05 was considered statistically significant.

## RESULTS

### Deslanoside-induced BR on the contractility of JSMF

Deslanoside exerted stimulatory effects on JSMF in NCS in a dose range of 5-160 μmol/L (Figure 1A).

Eight low and 8 high contractile states of jejunal smooth muscle were established as described in Materials and Methods. The contractility of JSMF in both low and high contractile states was statistically different from that of normal control (Figure 1). Deslanoside (20 μmol/L) was used in all the assays based on the fact that deslanoside-induced BR on jejunal contractility was observed in a dose range of 10-40 μmol/L. Deslanoside produced significant stimulatory effects (45.3% ± 4.0% *vs* 87.0% ± 7.8%, *P* < 0.01) on the contractility of JSMF in all 8 low contractile states (Figure 1B), and produced significant inhibitory effects (180.6% ± 17.8% *vs* 109.9% ± 10.8%, *P* < 0.01) on the contractility of JSMF in all 8

high contractile states (Figure 1C).

### Western blotting analysis

The PMLC in jejunum was significantly decreased in RLCS in comparison with that in NCS (100.0% ± 9.4% *vs* 78.1% ± 4.1%, *P* < 0.01), and was significantly increased at RHCS in comparison with that in NCS (100.0% ± 6.7% *vs* 139.2% ± 8.5%, *P* < 0.01) (Figure 2). Deslanoside significantly increased the PMLC in RLCS (78.1% ± 4.1% *vs* 96.0% ± 8.1%, *P* < 0.01), and significantly decreased the PMLC in RHCS (139.2% ± 8.5% *vs* 105.5 ± 7.34, *P* < 0.01).

### Effects of deslanoside on the contractility of JSMF in the presence of TTX

In the presence of TTX, BR was not observed when deslanoside was tested on the contractility of JSMF in RLCS and RHCS (Figure 3).

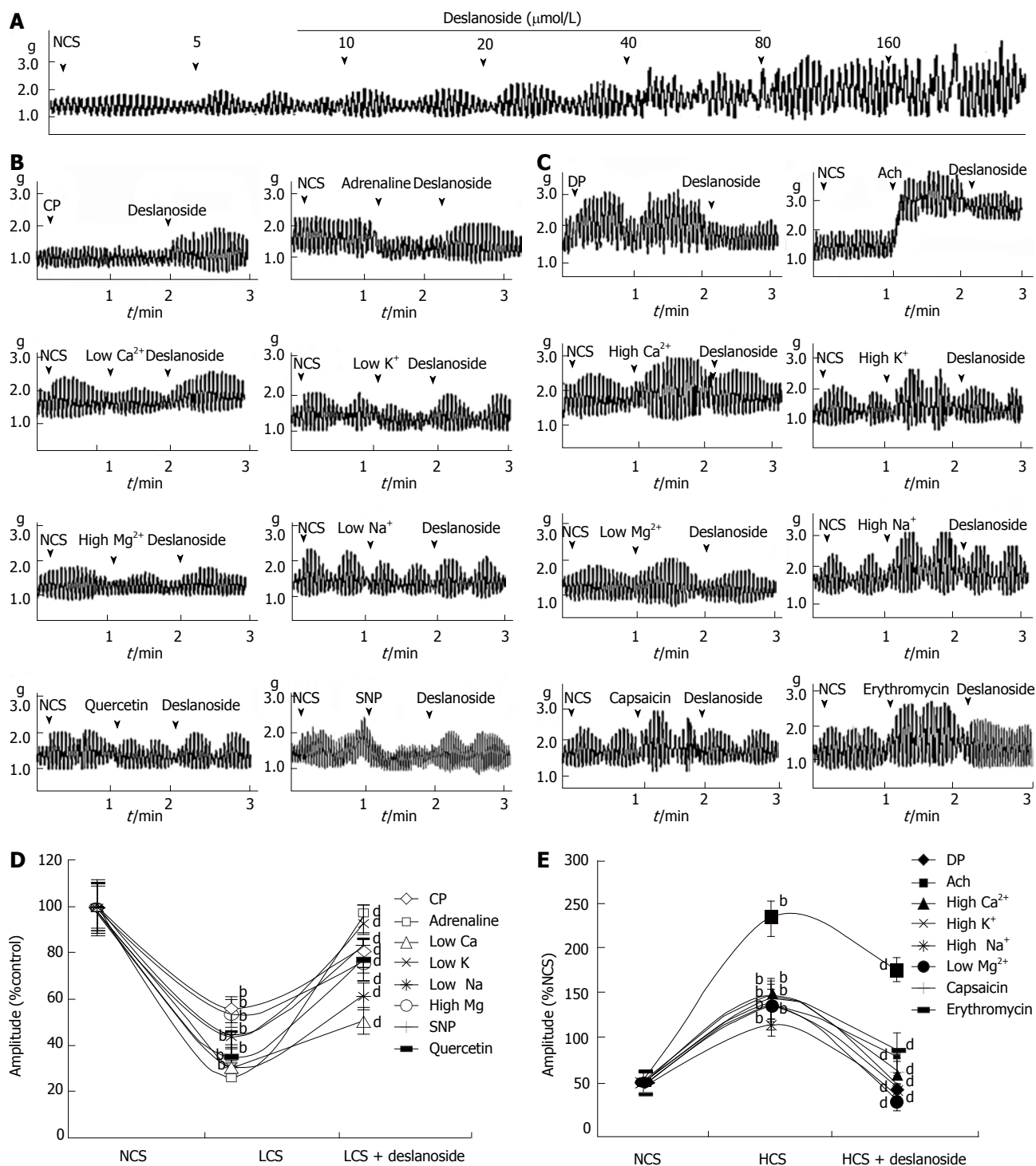
### Underlying mechanisms involved in deslanoside-induced BR

Deslanoside, at bath concentrations of 5 μmol/L, 20 μmol/L and 80 μmol/L, did not affect jejunal contractility in a Ca<sup>2+</sup>-free assay condition, and 20 μmol/L deslanoside did not stimulate the contractility of JSMF pre-incubated with the Ca<sup>2+</sup> channel blocker verapamil at normal, low and high contractile states (Figure 4).

The underlying mechanisms involved in deslanoside-induced BR were investigated. Muscarinic receptor antagonist atropine abolished the stimulatory effect of deslanoside on the contractility of JSMF in RLCS (Table 1; Figure 5A). Neither histamine H1-receptor antagonist diphenhydramine nor histamine H2-receptor antagonist cimetidine blocked deslanoside-induced stimulatory effects on the contractility of JSMF in RLCS (Table 1; Figure 5A). α-adrenergic receptor antagonist phentolamine, β-adrenergic receptor antagonist propranolol and NO synthase inhibitor L-NNA abolished deslanoside-induced inhibitory effect on the contractility of JSMF in RHCS (Table 1; Figure 5B).

## DISCUSSION

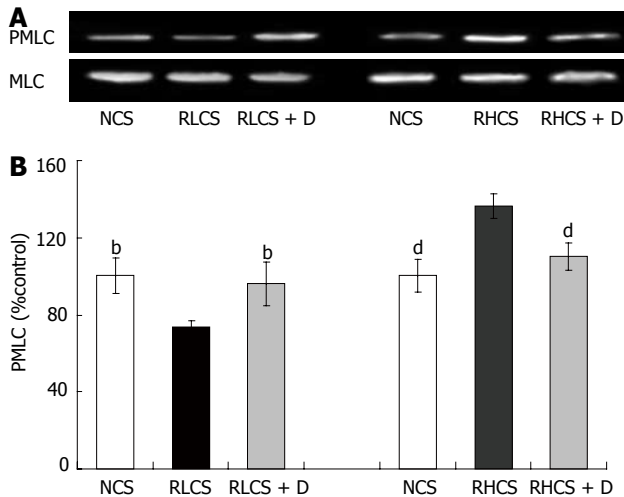
Eight pairs of low-high contractile states were established to imitate intestinal hyper- and hypomotility and



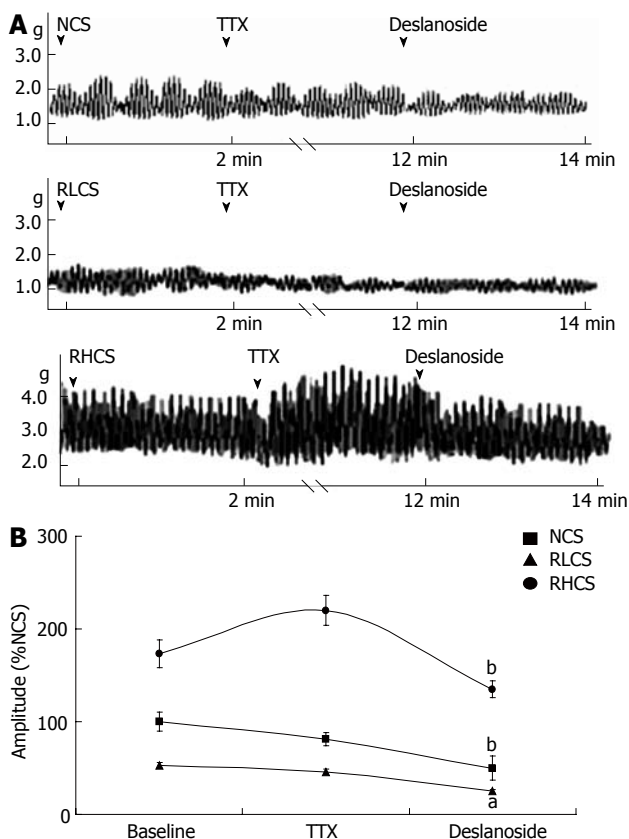
**Figure 1** Deslanoside-induced bidirectional regulation on the contractility of jejunal smooth muscle fragment. **A**: Dose response relationship of Deslanoside; **B**, **D**: Representative traces and statistical analysis of total traces from six independent experiments of Deslanoside-induced bidirectional regulation on the contractility of jejunal smooth muscle fragment (JSMF) in eight low contractile states (LCS); **C**, **E**: Representative traces and statistical analysis of total traces from six independent experiments of Deslanoside-induced bidirectional regulation on the contractility of JSMF in eight high contractile states (HCS). The median value of contractile amplitude of JSMF in normal contractile state is set to 100%, normal contractile state (NCS, control). Low and high contractile states of JSMF are the relative values compared with NCS. Data are expressed as the mean  $\pm$  SE (%NCS,  $n = 6$ ); <sup>a</sup> $P < 0.01$  vs the control; <sup>b</sup> $P < 0.01$  vs contractile amplitude of JSMF in LCS or HCS before Deslanoside administration. CP: Constipation-prominent rats; DP: Diarrhea-prominent rats; SNP: Sodium nitroprusside; Ach: Acetylcholine.

to evaluate the characteristics of Deslanoside-induced BR and potential clinical implication. IBS is known as one of the major functional gastrointestinal disorders, affecting approximately 10% of all adults worldwide<sup>[28]</sup>. IBS is usually categorized into three subclasses: IBS with

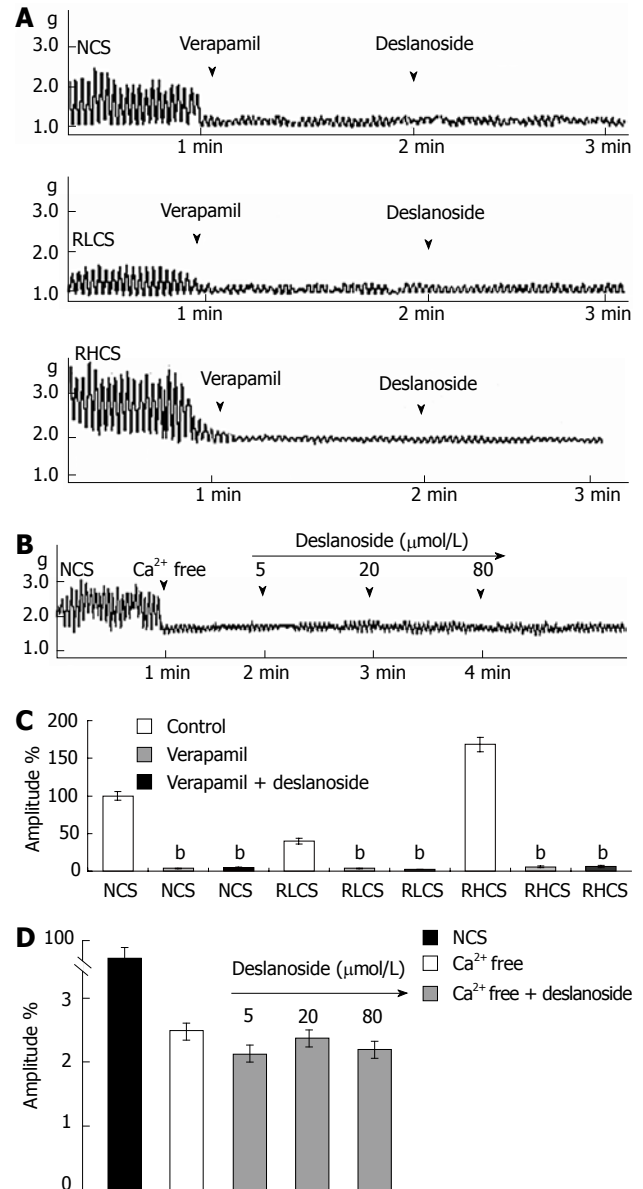
constipation (hypo-motility), IBS with diarrhea (hyper-motility), and IBS with alternating symptoms of both constipation and diarrhea (IBS-A)<sup>[13,14,29]</sup>. None of the currently available drugs are globally effective in treating all IBS symptoms<sup>[30]</sup>, and developing treatment strategies



**Figure 2** Western blotting analysis of the phosphorylation of myosin light chain. A: Representative images of Western blotting of the phosphorylation of myosin light chain (PMLC) of jejunum; B: Statistical analysis of band intensities of the PMLC in 4 independent experiments in normal contractile state (NCS, control), representative low contractile state (RLCS), and representative high contractile state (RHCS). To correct for loading variations, the result is expressed as a ratio of phosphor-myosin light chain to myosin light chain and NCS control is defined as 100%. Data represent mean  $\pm$  SE from 4 independent experiments; <sup>b</sup> $P < 0.01$  vs RLCS; <sup>d</sup> $P < 0.01$  vs RHCS. D: Deslanoside.

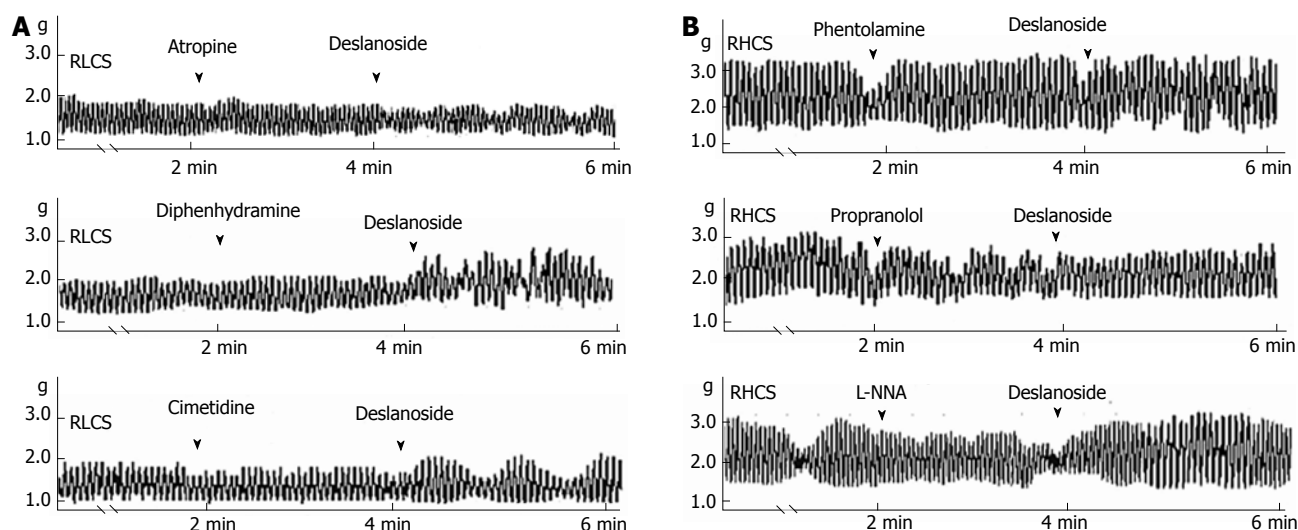


**Figure 3** Effects of deslanoside on the contractility of jejunal smooth muscle fragment pretreated with tetrodotoxin. A: Representative traces of deslanoside on the contractility of jejunal smooth muscle fragment (JSMF) pre-treated with tetrodotoxin in normal contractile state (NCS), representative low contractile state (RLCS) and representative high contractile state (RHCS); B: Statistical analysis obtained from independent assays. Other data are the relative values compared with NCS. Data are expressed as the mean  $\pm$  SE (%NCS,  $n = 6$ ); <sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$  vs contractile amplitude of JSMF after treatment with tetrodotoxin (TTX), (0.1  $\mu$ mol/L).



**Figure 4** Effects of deslanoside on the contractility of jejunal smooth muscle fragment in  $\text{Ca}^{2+}$ -free conditions. A: Representative traces of deslanoside (20  $\mu$ mol/L) on the contractility of jejunal smooth muscle fragment (JSMF) pre-treated with verapamil (0.1  $\mu$ mol/L) in normal contractile state (NCS), representative low contractile state (RLCS) and representative high contractile state (RHCS); B: Representative traces of deslanoside (5–80  $\mu$ mol/L) on the contractility of JSMF pre-treated with  $\text{Ca}^{2+}$ -free Krebs buffer; C, D: Statistical analysis obtained from 6 independent assays in identical assay conditions as (A) and as (B). The mean contractile amplitude of JSMF without drug treatment in NCS is set to a relative value of 100%, other data are expressed as mean  $\pm$  SE (% NCS,  $n = 6$ ); <sup>b</sup> $P < 0.01$  vs the control group.

for patients with IBS has been difficult because of the lack of pharmacological targets and the wide range of symptomatology<sup>[31]</sup>. Considering that the precise cause of IBS is unknown and it is unlikely that one single factor could explain all instances of IBS<sup>[32]</sup>, we established various assay conditions to mimic the possible intestinal hyper- and hypo-motility. These low and high contractile states of isolated intestinal smooth muscle were established (1) by changing ionic concentration in assay buffers; (2) using inhibitory and stimulatory neurotransmitters, or using exog-



**Figure 5** Effects of deslanoside on the contractility of jejunal smooth muscle fragment pretreated with receptor antagonist. A: Effects of deslanoside on the contractility of jejunal smooth muscle fragment (JSMF) pretreated with 10  $\mu\text{mol/L}$  atropine, 10  $\mu\text{mol/L}$  diphenhydramine and 10  $\mu\text{mol/L}$  cimetidine in the representative low contractile state (RLCS), respectively; B: Effects of deslanoside on the contractility of JSMF pretreated with 10  $\mu\text{mol/L}$  phentolamine, 5  $\mu\text{mol/L}$  propranolol and 10  $\mu\text{mol/L}$  L-NG-nitroarginine (L-NNA) in the representative high contractile state (RHCS), respectively.

enous inhibitors and stimulators in the assay buffers; and (3) using isolated intestinal smooth muscle obtained from constipation-prominent rat model and diarrhea-prominent rat model.

In this study, we tried to evaluate the possibility that whether deslanoside-induced adverse gastrointestinal irritation could be beneficialized as a potential therapeutic effect on the intestinal smooth muscle dysfunction, and characterized deslanoside-induced BR on the contractility of JSMF. Deslanoside was found to induce stimulatory effects on JSMF in all eight low contractile states and induced inhibitory effects on JSMF in all eight high contractile states. In accordance with deslanoside-induced BR on the contractility of JSMF, the effects deslanoside on myosin phosphorylation of JMSF were also bidirectional.

Activation of muscarinic receptor increases the intestinal motility and stimulation of  $\alpha$  and  $\beta$ -adrenoceptors inhibits intestinal motility. Inhibition of intestinal motility is also mediated by NO, a nonadrenergic, noncholinergic neurotransmitter, producing its effect by directly acting on smooth muscle and by indirectly inhibiting acetylcholine and substance P releasing<sup>[33,34]</sup>. Based on the aforementioned mechanisms involved in the modulation of intestinal contractility, our results have the following implications. The evidence that atropine blocked the stimulatory effects of deslanoside on JSMF in RLCS implies that stimulatory effects of deslanoside on JSMF in low contractile state are correlated with M receptor linked stimulation; and the evidence that phentolamine, propranolol and L-NNA abolished the inhibitory effects of deslanoside on JSMF in RHCS suggests that the inhibitory effects of deslanoside are correlated with adrenergic  $\alpha$ ,  $\beta$  receptor, as well as NO synthase linked relaxing mechanisms. Deslanoside-induced BR is  $\text{Ca}^{2+}$ -dependent, since it neither affected jejunal contractility in a  $\text{Ca}^{2+}$ -free assay condition, nor stimulated jejunal contractility pre-incubated with the  $\text{Ca}^{2+}$  channel blocker

verapamil in normal, low and high contractile states (Figure 4). The evidence that deslanoside-induced BR is not observed in the presence of TTX implies that deslanoside-induced BR is based on the presence of ENS.

Compared with controls ( $85.3 \pm 37.3$  min), the transit times (a measurement of bowel movement) obtained in constipation-predominant ( $67.4 \pm 19.6$  min) and diarrhea-predominant patients with IBS ( $108.4 \pm 34.3$  min) were decreased and increased, respectively ( $P < 0.05$ )<sup>[35]</sup>. The results implicate that deslanoside-induced BR on jejunum is informative for preclinical investigation of a drug with potential value for the modulation of both abnormally low and high contractility of intestinal smooth muscle. To relieve the symptoms of functional bowel disorders, such as alternating-type IBS, BR-inducer deslanoside could be considered for the potential future clinical application.

It is known that ENS is highly interconnected and responsible for secreting at least 50 different modulators, regulating intestinal motility and other functions<sup>[36]</sup>. We are still not clear about the diverse mechanisms for BR induction, including how dozens of neurotransmitters in intestinal smooth muscle are interrelated in normal contractile state, and how they correlate with BR in both the low and high contractile states. Although we have partially revealed the characteristics of deslanoside-induced BR, further study is still required to identify the detailed mechanisms.

## ACKNOWLEDGMENTS

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## COMMENTS

### Background

Irritable bowel syndrome (IBS) is known as one of major functional gastro-

intestinal disorders, contracting approximately 10% of all adults world wide. Cardiotonic glycosides (CGs) have long been and continue to be used in the treatment of congestive heart failure and have entered clinical trials for treating cancer. Gastrointestinal irritation of CGs has been reported, however, the characteristics of CGs on intestinal motility remain unknown.

### Research frontiers

Developing treatment strategies for patients with IBS has been difficult because of the lack of pharmacological targets and the wide range of symptomatology, especially in the alternating-type IBS (IBS-A) which is a functional gastrointestinal disorder with alternating symptoms of both constipation and diarrhea.

### Innovations and breakthroughs

The present study established 8 pairs of low-high contractile states to mimic the possible intestinal smooth muscle disorders. These different low and high contractile states of isolated intestinal smooth muscle were established by changing ionic concentration in assay buffers; using inhibitory and stimulatory neurotransmitters; exogenous inhibitors and stimulators, respectively in the assays; and isolated intestinal smooth muscle obtained from both constipation-prominent rat model and diarrhea-prominent rat model. The results indicate that the contractile state determines deslanoside-induced effects to be stimulatory or inhibitory, namely, stimulatory effects on the contractility of intestinal fragment were induced by deslanoside in all low contractile states, and inhibitory effects were induced on the contractility of jejunal smooth muscle fragment (JSMF) in all high contractile states. The present study indicates that deslanoside-induced Bidirectional regulation (BR) requires the presence of enteric nervous system and is  $\text{Ca}^{2+}$  dependent. The possible mechanism of deslanoside-induced BR is related to cholinergic system when jejunal smooth muscle is in a low contractile state, and related to adrenergic system and nitric oxide relaxing mechanism when in a high contractile state.

### Applications

The results implicate that deslanoside-induced BR on jejunum is informative for preclinical investigation of a drug with potential value for the modulation of both abnormally low and high contractility of intestinal smooth muscles. To relieve the symptoms of functional bowel disorders, such as IBS-A, BR-inducer deslanoside could be considered for the potential future clinical application.

### Terminology

IBS is usually classified into three subclasses: IBS with constipation (hypomotility), IBS with diarrhea (hyper-motility), and IBS with alternating symptoms of both constipation and diarrhea.

### Peer review

This is a well done study that provides interesting insight into the action of deslanoside. The study is complete, well-written and suitable for publication.

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