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BRIEF ARTICLE

HEF-19-induced relaxation of colonic smooth muscles and the underlying mechanisms

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Abstract

AIM: To investigate the relaxant effect of chromane HEF-19 on colonic smooth muscles isolated from rabbits, and the underlying mechanisms.

METHODS: The relaxant effect and action mechanisms of HEF-19 were investigated using descending colon smooth muscle of the rabbits. Preparations 1 cm long were mounted in 15-mL tissue baths containing Tyrode's solution, maintained at 37 ± 0.5 ℃ and aerated with a mixture of 5% CO_2 in oxygen (Carbogen). The tension and amplitude of the smooth muscle strips were recorded after adding HEF-19 (10^{-6}, 10^{-5} and 10^{-4} mol/L). After cumulative administration of four antispasmodic agents, including acetylcholine chloride (Ach) (10^{-4} mol/L), histamine (10^{-4} mol/L), high-K^+ (60 mmol/L) and $BaCl_2$ (8.2 mmol/L), HEF-19 (3×10^{-7}-3×10^{-4} mol/L) was added to investigate the relaxant effect of HEF-19. $CaCl_2$ (10^{-4}-2.5×10^{-3} mol/L) was added cumulatively to the smooth muscle preparations pretreated with and without HEF-19 (1×10^{-6} or 3×10^{-6} mol/L) and verapamil (1×10^{-7} mol/L) to study the mechanisms involved. Finally, phasic contraction was induced with ACh (15×10^{-6} mol/L), and $CaCl_2$ (4×10^{-3} mol/L) was added to the smooth muscle preparations pretreated with and without HEF-19 (3×10^{-6} mol/L or 1×10^{-5} mol/L) and verapamil (1×10^{-7} mol/L) in calcium-free medium to further study the underlying mechanisms.

RESULTS: HEF-19 (1×10^{-6}, 1×10^{-5} and 1×10^{-4} mol/L) suppressed spontaneous contraction of rabbit colonic smooth muscles. HEF-19 (3×10^{-7}-3×10^{-4} mol/L) relaxed in a concentration-dependent manner colonic smooth muscle preparations pre-contracted with $BaCl_2$, high-K^+ solution, Ach or histamine with respective EC_{50} values of 5.15 ± 0.05, 5.12 ± 0.08, 5.58 ± 0.16 and 5.25 ± 0.24, thus showing a spasmolytic activity. HEF-19 (1×10^{-6} mol/L and 3×10^{-6} mol/L) shifted the concentration-response curves of $CaCl_2$ to the right and depressed the maximum response to $CaCl_2$. The two components contracted by Ach were attenuated with HEF-19 (3×10^{-6} mol/L or 10^{-5} mol/L) in calcium-free medium.

CONCLUSION: HEF-19 inhibited rabbit colonic smooth muscle contraction, probably through inhibiting opening of voltage-dependent Ca^{2+} channels. HEF-19 reduced inflow and intracellular release of Ca^{2+} ions.

Key words: Colonic smooth muscle; Smooth muscle relaxation; Ca^{2+} channels

Core tip: This is a good descriptive study in which authors found a new L-calcium-antagonist relaxing rabbit colonic smooth muscles and analyzed its possible mechanism. It provides an opportunity to search for a new drug highly selective to the gastrointestinal tract, effectively relieving pain, diarrhea and intestinal discomfort, but without significant adverse effects on irritable bowel syndrome patients.

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INTRODUCTION

Irritable bowel syndrome (IBS) is a frequent gastrointestinal disease, characterized by a combination of several symptoms including abdominal pain or discomfort, flatulence, and problems related to bowel habits (constipation and/or diarrhea)[1]. Abnormal contraction of intestinal smooth muscle may be importantin producing the main IBS symptoms. thus, modifying the contractility is often the major aim in the treatment of IBS[2,3]. Calcium channel blockers have a good effect on IBS patients with abdominal pain and diarrhea[4].Calcium channel blockers have received increasing attention in the treatment of IBS. 3.4-Dihydro-7-[3-(diethylamino) propoxy] chroman hydrochloride (HEF-19) is a compound with a relaxant effect on colonic smooth muscles.

The present study investigated the relaxant effect of HEF-19 on isolated descending colon smooth muscle from rabbits, and the underlying mechanisms (Figure 1).

MATERIALS AND METHODS

Animals

New Zealand rabbits of either sex (2.0-2.5 kg) were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Certificate number: SCXK20030011). All care and handling of animals were approved by the Institutional Animal Ethical Committee.

Chemicals and reagents

Normal Tyrode's solution contained: NaCl 136.86 mmol/L, KCl 2.68 mmol/L, $NaHCO_3$ 11.9 mmol/L, $MgCl_2$ 1.05 mmol/L, KH_2PO_4·H_2O 0.41 mmol/L, $CaCl_2$ 1.8 mmol/L, and glucose 5.6 mmol/L. A high-K^+ solution (KCl, 60 mmol/L) was obtained by equimolar replacement of NaCl by KCl in Tyrode's solution[5]. Ca^{2+}-free Tyrode solution was the solution in which $CaCl_2$ was omitted and ethylenediaminetetra-acetic acid (EDTA, 0.1 mmol/L) was added[6]. Ca^{2+}-free high-K^+ solution was the Ca^{2+}-free and high-K^+ Tyrode solution. All chemicals were dissolved in distilled water. All solutions were stored at 4 ℃ and fresh dilutions were made daily.

HEF-19 (> 99.5% purity) was provided by Organic Chemistry Laboratory of Shenyang Pharmaceutical University and dissolved in distilled water. KCl was from Shenyang Chemical Reagent Factory, Shenyang, China, $CaCl_2$ from Tianjin Bodi Chemical Co., Tianjin, China, $BaCl_2$ from Shenyang Xingdong Reagent Factory, Shenyang, China, verapamil injection from Tianjin Heping Pharmaceutical Plant, Tianjin, China, and acetylcholine chloride (Ach) and histamine were from Sigma, United States.

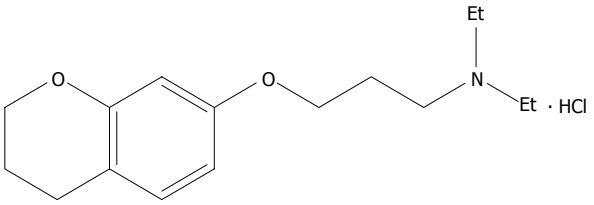

Figure 1 3.4-dihydro-7-[3-(diethylamino) propoxy] chroman hydrochloride.

Preparation of colonic smooth muscles

The animals had free access to water but were fasted for 24 h before the experiments. The animals were killed by a blow to the head. The descending colon portion was isolated, washed, and freed from the mesentery. Preparations 1 cm long were mounted in 15-mL tissue baths containing Tyrode's solution maintained at 37 ± 0.5 ℃ and aerated with a mixture of 5% CO_2 in oxygen. A preload of 3 g was applied and the tissues were kept undisturbed for an equilibrium period of 60 min. During that time, the nutrient solution was changed every 20 min. Changes in isometric tension were measured with a force-displacement transducer (Chengdu Instrument Plant, Chengdu, China) and recorded by an RM6240B Multichannel Physiological Signal Collection and Handling System (Chengdu Instrument Plant)[7].

Effect of HEF-19 on spontaneous contraction of rabbit descending colon

The normal tension and amplitude of the descending colonic smooth muscle strips were recorded after the contraction reached a stable plateau. HEF-19 (1×10^{-6}, 1×10^{-5} and 1×10^{-4} mol/L) and vehicle were added to the tissue baths containing Tyrode's solution.

Relaxant effect of HEF-19 on contraction induced by $BaCl_2$, high-K^+ solution, Ach or histamine

The isolated colon smooth muscle preparations were contracted with Ach (1×10^{-4} mol/L), histamine (1×10^{-4} mol/L) High-K^+ (60 mmol/L) or $BaCl_2$ (8.2 mmol/L), after the contraction reached a stable plateau, and cumulative concentrations of HEF-19 (3×10^{-7} mol/L-3×10^{-4} mol/L) were added. The relaxant effect was expressed as a percentage of relaxation and the EC_{50} (concentration to produce a 50% maximal relaxation) was calculated using a multichannel physiological system.

Inhibition of $CaCl_2$-induced cumulative contractions

The isolated preparations were allowed to stabilize in normal Tyrode's solution and were replaced with Ca^{2+}-free Tyrode's solution for 30 min, and then K^+-rich and Ca^{2+}-free Tyrode's solution. After 15 min incubation, Ca^{2+} was added in a cumulative fashion (1×10^{-4}-2.5×10^{-3} mol/L) to obtain control concentration-response curves. The results were expressed as the percentage of the maximum contractile tension to $CaCl_2$ before and after pretreatment

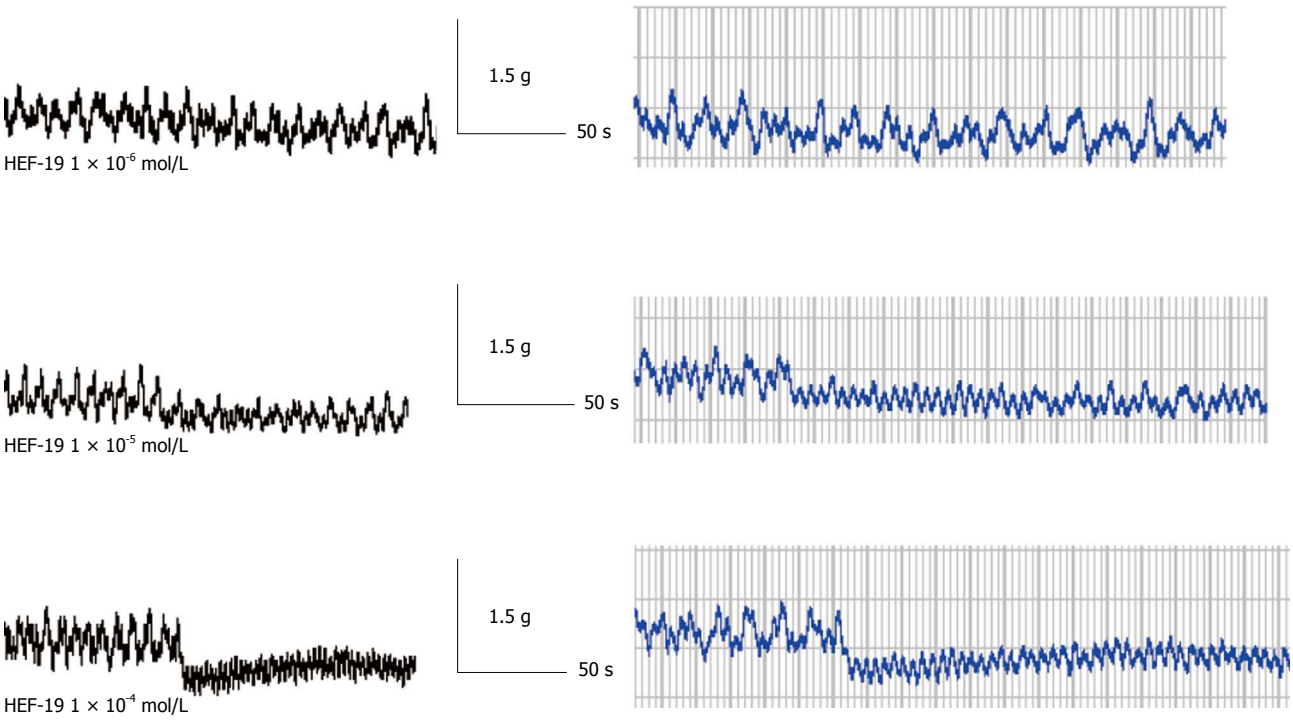

Figure 2 Effects of HEF-19 on spontaneous tension and amplitude of isolated rabbit descending colonic smooth muscle.

Table 1 Effects of HEF-19 on tension and amplitude of spontaneous contraction of descending colonic smooth muscles

Group	After administration (%)	
	Tension	Amplitude
Vehicle	97.98 ± 2.37	103.69 ± 10.13
HEF-19 (mol/L)		
10^{-6}	89.87 ± 2.60	81.96 ± 13.90[a]
10^{-5}	75.98 ± 3.2[b]	48.40 ± 6.07[b]
10^{-4}	55.05 ± 18.13[b]	37.77 ± 2.54[b]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* vehicle. Values are mean ± SD, $n = 6$.

with HEF-19 (1×10^{-6} or 3×10^{-6} mol/L) and verapamil (1×10^{-7} mol/L) respectively[8].

Inhibition of HEF-19 on biphasic contraction induced by ACh

After the equilibration period, normal Tyrode's solution was replaced with Ca^{2+}-free Tyrode's solution for 20 min. The phasic contraction caused by ACh (15×10^{-6} mol/L) was obtained, and tonic contraction was induced by further addition of $CaCl_2$ (4×10^{-3} mol/L). After washing with normal Tyrode's solution, the experiments were repeated with incubation for 10 min with HEF-19 (3×10^{-6} mol/L or 1×10^{-5} mol/L) and verapamil (1×10^{-7} mol/L) respectively[8,9].

Statistical analysis

Statistical evaluation of the data was performed using Student's *t* test when appropriate. The data were expressed as mean ± SD or mean ± SEM and $P < 0.05$ was considered statistically significant.

RESULTS

Effect of HEF-19 on spontaneous contraction of rabbit descending colon

HEF-19 (1×10^{-6}, 1×10^{-5} and 1×10^{-4} mol/L) significantly suppressed the tension and amplitude of *spontaneous contraction*, in a concentration-dependent manner. Figure 2 is print screen about tension and amplitude of spontaneous contraction of descending colonic smooth muscles. Tension is *y*-axis. Time is *x*-axis. Amplitude is difference between the peaks and troughs. The data of Figure 2 showed in Table 1.

Relaxant effects of HEF-19 in contraction induced by $BaCl_2$, high-K^+ solution, Ach or histamine

The maximum responses of the cumulative concentration-response curves to $BaCl_2$, high-K^+ solution, Ach or histamine were depressed by HEF-19 in a dose-dependent manner (3×10^{-7}-3×10^{-4} mol/L). EC_{50} values were 5.15 ± 0.05, 5.12 ± 0.08, 5.58 ± 0.16 and 5.25 ± 0.24 (Figure 3).

Inhibition of $CaCl_2$-induced contraction

The maximum cumulative concentration-response curves for $CaCl_2$-induced contraction were depressed by HEF-19 (1×10^{-6} and 3×10^{-6} mol/L) in a concentration-dependent manner. These results indicated that HEF-19 showed non-competitive antagonism (Figure 4).

Inhibitory effect of HEF-19 on biphasic contraction induced by ACh

The phasic and tonic contraction induced by ACh was decreased by HEF-19 (3×10^{-6} and 1×10^{-5} mol/L) in a

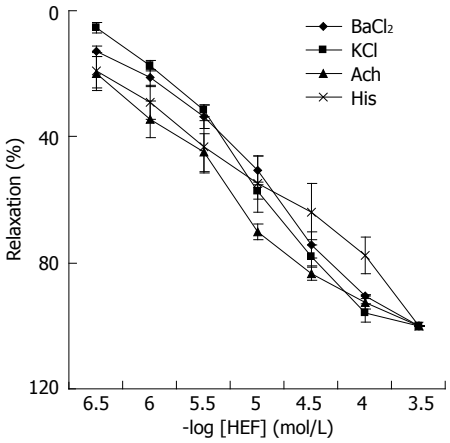

Figure 3 Relaxant effect of HEF-19 (3×10^{-7}-3×10^{-4} mol/L) on isolated rabbit descending colonic smooth muscle pre-contracted with Ach, histamine, high-K^+ solution or $BaCl_2$. Data are mean ± SE (n = 6).

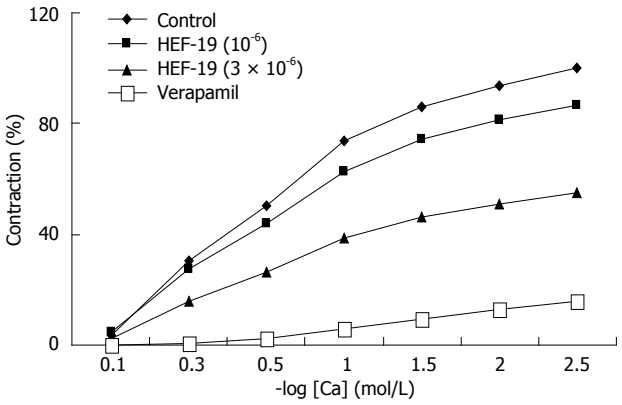

Figure 4 Effect of HEF-19 and verapamil on the contraction-response curve of $CaCl_2$ in descending colonic smooth muscle isolated from rabbits. Data are mean ± SE (n = 6) and are expressed as percentage of maximum contraction.

concentration-dependent manner after pretreatment in calcium-free medium with EGTA (Figure 5).

DISCUSSION

Excitation-contraction coupling in smooth muscle occurs through two main mechanisms. Many smooth muscles are activated by Ca^{2+} signaling cascades. In addition, there is a Rho/Rho kinase signaling pathway that acts by altering the Ca^{2+} sensitivity of the contractile system[10,11]. The predominant source of activator and intracellular Ca^{2+} has little role to play in mediating excitation-contraction coupling by agonists. Both tonic and phasic (rhythmic) contraction are regulated by intracellular Ca^{2+} concentration. Ca^{2+} originates from the intracellular Ca^{2+} store, the sarcoplasmic reticulum, and influx from the extracellular space. Phasic contraction is influenced by neurotransmitters, hormones, and drugs. In circular muscle, these agents can also increase calcium by releasing it from intracellular stores, thus inducing tonic contraction[12-19].

Smooth muscle has the automatic rhythmicity. Spontaneous contraction shows the basic rhythmic depolarization wave. HEF-19 suppressed the spontaneous contractile amplitude and tension of rabbit colonic smooth muscle in a concentration-dependent manner. It has been reported that extracellular Ca^{2+} participates in spontaneous activity and enters the cytosol by L-type voltage-dependent Ca^{2+} channels[20].

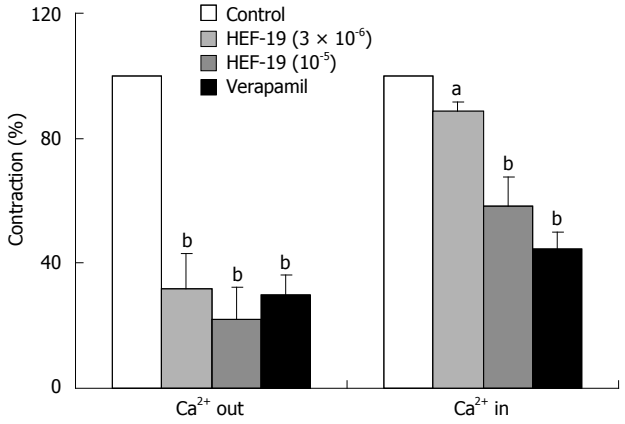

Figure 5 Effects of HEF-19 (3×10^{-6} and 10^{-5} mol/L) and verapamil (1×10^{-7} mol/L) on biphasic contraction induced by Ach in descending colonic smooth muscle isolated from rabbits. Data are mean ± SE (n = 6). $^aP < 0.05$, $^bP < 0.01$ *vs* the controls.

The contraction induced by $BaCl_2$, high-K^+ solution, Ach or histamine was relaxed by HEF-19. High-K^+ elicits an increase in intracellular Ca^{2+} and transient contractions[21,22]. ACh induces smooth muscle contraction via activating muscarinic receptors. Extracellular and intracellular Ca^{2+} participate in the Ach-induced contraction[23]. Histamine has a spasmogenic effect on the gastrointestinal tract through activating histaminergic receptors and increasing Ca^{2+} influx[24,25]. $BaCl_2$ causes cell membrane depolarization and intracellular Ca^{2+} release, and it can cross the cell membrane through the Ca^{2+} channels to bind with troponin directly[26].

HEF-19 depressed the maximum cumulative concentration-response curve for $CaCl_2$ in a non-competitive manner, similar to verapamil. The fact that HEF-19 inhibited $CaCl_2$-induced smooth muscle contraction indicated that it inhibited the voltage-dependent Ca^{2+} channels, because $CaCl_2$ can open these channels during high-K^+ depolarization[27,28].

There are biphasic responses, including fast and slow components, in the contraction induced by ACh. The fast (phasic) phase is due to the release of intracellular Ca^{2+} induced by ACh in Ca^{2+}-free medium[21], and the sustained (tonic) phase is largely dependent on the influx of external Ca^{2+} resulting from the reintroduction of $CaCl_2$ into the medium. HEF-19 decreased the phasic and tonic contraction. The results showed that HEF-19 eventually inhibited the Ca^{2+} channels to reduced release of intracellular Ca^{2+} and influx of external Ca^{2+}.

In conclusion, our results suggest that HEF-19 relaxed rabbit descending colonic smooth muscle by blocking voltage-dependent Ca^{2+} channels. HEF-19 inhibited

the inflow of extracellular Ca^{2+} into cells, and intracellular release of Ca^{2+} ions. Ca^{2+} channels blocking effect of HEF-19 is fewer than verapamil on colonic smooth muscle. Calcium channel blockers are also reported to be effective in the treatment of IBS[3]. However, the adverse effects on the cardiovascular system of these blockers limit their further application on IBS patients. HEF-19, a L-type calcium channel blocker with selectivity for the gastrointestinal tract, is expected to be a safe and effective drug for treatment of abdominal pain and diarrhea symptoms associated with IBS.

COMMENTS

Background

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder in which abdominal pain is associated with changes in bowel habits and abdominal distension. Abnormal contraction of intestinal smooth muscle may be important in producing the main symptoms of IBS, thus, modifying contractility is often the major aim of treatment. Traditional cholinolytic and opioid drugs have been reported to have much adverse reactions. Some enteric spasmolytics agents have been found to treat IBS by selectively blocking voltage-dependent Ca^{2+} channels.

Research frontiers

Current IBS pathophysiologic mechanisms are based on the abnormalities of brain-gut axis. With in-depth researches on various neurotransmitters, ion channel and receptors, designed as targets, new drugs are expected to appear against IBS. Since Pinaverium Bromide was developed and used clinically, there has been increasing concern to search for highly selective blockers of voltage-dependent Ca^{2+} channels to treat IBS patients with abdominal pain and diarrhea.

Innovations and breakthroughs

Chromane HEF-19 has a relaxant effect on colonic smooth muscles. It has previously been shown to have little activity on isolated vascular smooth muscle. The present study investigated the relaxant effect of HEF-19 on isolated descending colon smooth muscle of rabbits and the possible mechanisms. HEF-19 is expected to be a highly selective enteric spasmolytics agent through inhibition of opening of voltage-dependent Ca^{2+} channels in colonic smooth muscle. This is a potentially interesting study to find a drug for treatment of abdominal pain and diarrhea associated with IBS.

Applications

HEF-19 is expected to be a safe, effective and economic drug for treatment of abdominal pain and diarrhea symptoms associated with IBS.

Terminology

HEF-19: HEF-19, 3.4-dihydro-7-[3-(diethylamino) propoxy] chroman hydrochloride, is highly selective enteric spasmolytics agent. IBS is a functional gastrointestinal disorder in which abdominal pain is associated with changes in bowel habits and abdominal distension. People with a functional gastrointestinal (GI) disorder have frequent symptoms, but the GI tract is not damaged. IBS is a group of symptoms that occur together. The most common symptoms of IBS are abdominal pain or discomfort, often reported as cramping, along with diarrhea, constipation, or both.

Peer review

Very well written manuscript. In the manuscript entitled "HEF-19-induced relaxation of colonic smooth muscles and the underlying mechanisms", the authors investigated the relaxant effect of chromane HEF-19 on colonic smooth muscles isolated from rabbits. This is a good descriptive study on a hot topic. The research is well done. The result is well discussed.

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