

Activation of neural cholecystokinin-1 receptors induces relaxation of the isolated rat duodenum which is reduced by nitric oxide synthase inhibitors

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Abstract

Cholecystokinin (CCK) influences gastrointestinal motility, by acting on central and peripheral receptors. The aim of the present study was to determine whether CCK has any effect on isolated duodenum longitudinal muscle activity and to characterize the mechanisms involved. Isolated segments of the rat proximal duodenum were mounted for the recording of isometric contractions of longitudinal muscle in the presence of atropine and guanethidine. CCK-8S (EC₅₀: 39; 95% CI: 4.1-152 nM) and cerulein (EC₅₀: 58; 95% CI: 18-281 nM) induced a concentration-dependent and tetrodotoxin-sensitive relaxation. N^onitro-L-arginine (L-NOARG) reduced CCK-8S- and cerulein-induced relaxation (IC₅₀: 5.2; 95% CI: 2.5-18 μM) in a concentration-dependent manner. The magnitude of 300 nM CCK-8S-induced relaxation was reduced by 100 μM L-NOARG from 73 ± 5.1 to 19 ± 3.5% in an L-arginine but not D-arginine preventable manner. The CCK-1 receptor antagonists proglumide, lorglumide and devazepide, but not the CCK-2 receptor antagonist L-365,260, antagonized CCK-8S-induced relaxation in a concentration-dependent manner. These findings suggest that CCK-8S and cerulein activate intrinsic nitrergic nerves acting on CCK-1 receptors in order to cause relaxation of the rat duodenum longitudinal muscle.

Key words

- CCK
- CCK receptor subtype 1
- Proximal duodenum
- Myenteric neurons
- Nitric oxide

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Introduction

Cholecystokinin (CCK) is produced and released by endocrine cells of the duodenal mucosa as well as by central and peripheral neurons including some primary afferent neurons innervating the gastrointestinal tract (1). Considerable evidence has accumulated indicating that CCK, in addition to exerting its well-known effects on pancreatic exo-

crine secretion, also regulates gastrointestinal motility (2-4). CCK interacts with two distinct receptors, type A or type B (5), now denominated type 1 and type 2 (6). Both receptor types have been implicated in the excitatory and inhibitory motor effects of CCK on the various regions of the gastrointestinal tract including the lower esophageal sphincter, pyloric muscle and small intestine (7-9). Although the presence of CCK recep-

tors in enteric neurons has been inferred from pharmacological studies and electrophysiological observations (10,11) and more recently from immunohistochemical studies of c-fos induction (12,13), direct evidence for the presence of CCK-A (type 1) receptors in rat myenteric neurons has been provided only recently (14,15). It has been suggested that CCK inhibits canine intestinal circular muscle through the activation of enteric nitrergic and/or vasoactive intestinal peptide-containing neurons (2). Therefore, the objectives of the present study were to determine whether CCK and cerulein, a CCK receptor agonist derived from frog skin, have any influence on isolated rat duodenal longitudinal muscle contractility and to characterize the sites of their action as well as the mechanisms involved.

Material and Methods

All procedures were conducted according to the local Ethics Guidelines for Animal Experimentation (Comissão de Ética em Experimentação Animal, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo) based on international guidelines of animal care and all efforts were made to minimize the number of animals used and their suffering. Male Wistar rats weighing 250-300 g were exsanguinated by section of the cervical vessels and a 2.0- to 2.5-cm long whole segment of the proximal portion of the duodenum was removed. The segments were mounted for the recording of the activity of longitudinal muscle under 2 g of resting tension in a 10-mL organ bath containing Ca^{2+} -free Tyrode solution (137 NaCl, 3 mM KCl, 1 mM MgCl_2 , 12 mM NaHCO_3 , 0.4 mM NaHPO_4 , and 11.1 mM glucose) maintained at 32°C and continuously bubbled with air. Longitudinal muscle tension was recorded polygraphically with a Hewlett-Packard 7754A polygraph (Palo Alto, CA, USA) by means of an isometric force transducer (FTA 10) as described (16). The duode-

nal segments were washed every 15 min for up to 120 min with Ca^{2+} -free Tyrode solution. Tension was then induced by adding CaCl_2 to the bath (1 mM final concentration). Under these conditions the tonus induced was stable and the spontaneous activity was of low amplitude. Because gastrointestinal motility inhibition is mediated mainly by nonadrenergic, noncholinergic neural mechanisms the effect of two CCK receptor agonists, CCK-8S and cerulein, was determined in the presence of 1 μM atropine and 3 μM guanethidine. Electrical field stimulation was applied through two ring platinum electrodes placed immediately above and below the duodenal segment, and consisted of square wave pulses (32 V, 0.5 ms, 1-8.0 Hz) applied for 10 s at 2- to 3-min intervals. Cerulein and CCK-8S concentration response curves were constructed by the non-cumulative method with 30-min intervals between each addition in order to prevent tachyphylaxis. The effect of the different inhibitors was determined in the same preparation after obtaining consistent control responses. Separate preparations were employed for testing tetrodotoxin (TTX), N^{ω} -nitro-L-arginine (L-NOARG) and each of the antagonists, which were incubated for at least 20 min before adding Ca^{2+} to raise the tension of the preparation.

Drugs

Cerulein, CCK-8S, TTX, atropine sulfate, guanethidine, L-NOARG, L-arginine (L-ARG), D-arginine (D-ARG), proglumide, and lorglumide were obtained from Sigma/RBI (St. Louis, MO, USA). Devazepide (L-364,718) and L-365,260 were from Merck & Co., Rahway, NJ, USA. Concentrated stock solutions of all drugs were prepared in distilled water except lorglumide, devazepide and L-365,260 which were dissolved in methanol. The amount of methanol added to the organ bath never exceeded 0.1% of the total volume. Drug- or nerve-elicited re-

sponses were not affected by the presence of this amount of methanol.

Statistical analysis

The EC_{50} values were calculated after logit-log transformation of the data and are reported as the geometric mean and 95% confidence intervals (95% CI). Relaxation is reported as percent reduction in tension or as percent of the control value obtained before adding the antagonists and is reported as mean \pm SEM. Statistical significance was determined by the Student *t*-test for paired samples, with the level of significance set at $P < 0.05$.

Results

Cerulein caused an initial rapid decrease in tension of the longitudinal muscle of the rat proximal duodenum followed by an increase in tension above the initial tonus; this biphasic response elicited by cerulein was abolished by 1 μ M TTX, which also abolished electrical field stimulation-elicited responses (Figure 1A). Similar findings were observed with CCK-8S (data not shown). The magnitude of relaxation increased with the concentration of both agonists (Figure 1B) and the potency of CCK-8S (EC_{50} : 39; 95% CI: 4.1-152 nM) was slightly higher but not statistically different from that of cerulein (EC_{50} : 58; 95% CI: 18-281 nM).

The selective CCK-1 receptor antagonists proglumide, lorglumide and devazepide, but not the CCK-2 receptor antagonist L-365,260, reduced the magnitude of 330 nM CCK-8S-induced relaxations in a concentration-dependent manner but had no effect, or even magnified the excitatory response (Figure 2).

The magnitude of 300 nM CCK-8S- and cerulein- (data not shown) elicited relaxation was reduced by L-NOARG in a concentration-dependent manner (IC_{50} : 5.2; 95% CI: 2.5-18 μ M), L-ARG (1 mM), but not D-

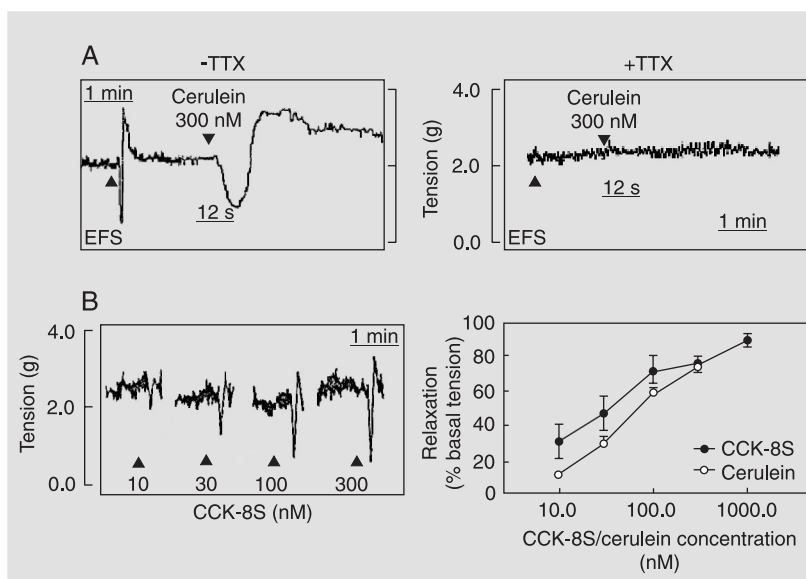


Figure 1. A, Representative tracings showing the effects of EFS (triangles) and cerulein (inverted triangles) on the tension of longitudinal muscle of rat duodenum in the absence (left) and in the presence (right) of 1 μ M tetrodotoxin (TTX). B, Left, Responses of rat duodenum longitudinal muscle induced by different concentrations of CCK-8S. The interval between the additions to the bath was 30 min. Right, Relationship between CCK-8S and cerulein concentrations and the magnitude of the relaxations of rat duodenum longitudinal muscle. Data are reported as the means \pm SEM for 4-6 experiments. EFS = electrical field stimulation; CCK-8S = cholecystokinin-8S.

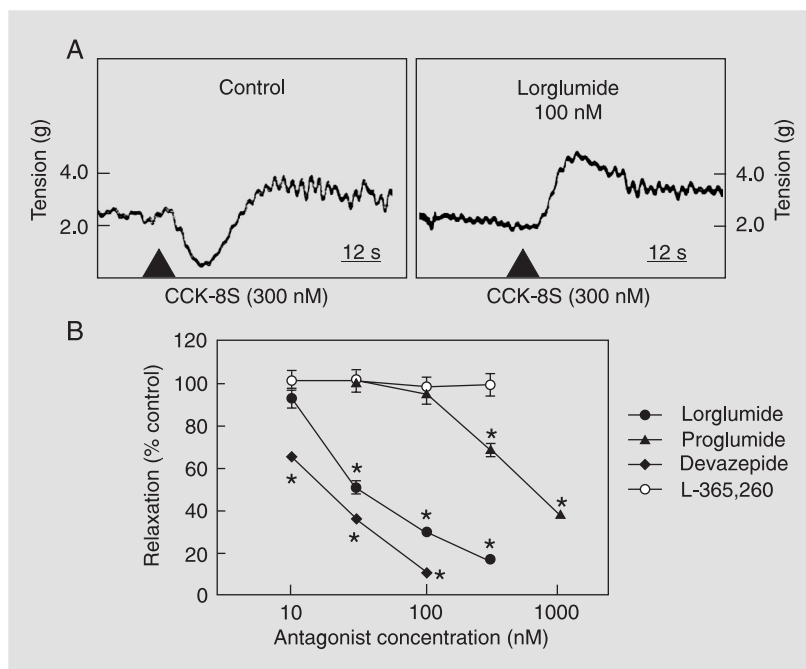


Figure 2. A, Representative tracings showing the effect of 300 nM CCK-8S on the tension of rat duodenum longitudinal muscle in the absence (left, control) and in the presence (right) of 100 nM lorglumide. B, Concentration dependence of different CCK antagonists on the magnitude of CCK-8S-induced relaxation of the rat duodenum longitudinal muscle. Data are reported as the means \pm SEM for 6-8 experiments. CCK-8S = cholecystokinin-8S. * $P < 0.05$ compared to control responses (paired Student *t*-test).

ARG (1 mM), prevented the reduction of CCK-8S-induced relaxation caused by 100 μ M L-NOARG (Figure 3).

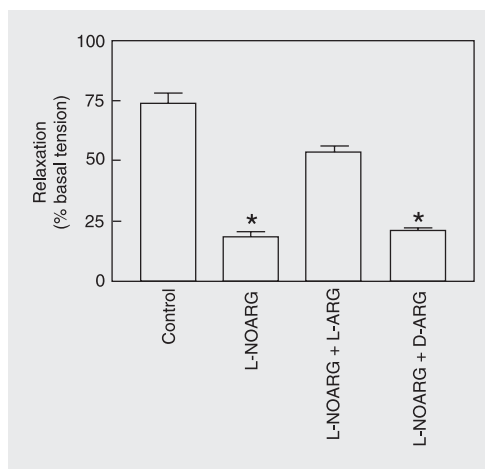
Discussion

The present study shows that CCK-8S and cerulein cause an initial relaxation followed by contraction of the isolated longitudinal muscle of the rat proximal duodenum. Both the excitatory and inhibitory actions of CCK on gastrointestinal motility have been previously described in intact dogs (2) and rats (3,4). The fact that in the present experiments both effects were abolished by TTX indicates that they are mediated by the activation of neural structures located in the duodenal wall. Although it has been reported that the *in vitro* contractile effect of CCK on gastrointestinal muscle is mediated in part by a presynaptic action of CCK involving the release of ACh from cholinergic intrinsic nerves (17), it is worth noting that the contractile response in our experiments was observed in the presence of atropine. Therefore, and in contrast with the findings in canine gastrointestinal tract (18,19), it is unlikely that the neural-dependent contractile effect of CCK on the longitudinal muscle of the rat proximal duodenum involves the activation of muscarinic receptors. Rather, this TTX-sensitive contraction probably depends on the release of substance P from

intrinsic nerves, as observed in the longitudinal muscle of the guinea pig ileum (20). Interestingly, CCK-1 receptors have been recently detected in a subpopulation of substance P-containing myenteric neurons, which is consistent with the possibility that the contractile effect of CCK-8S may involve release of substance P. However, since CCK-1 receptor antagonists did not affect the contractile response and even enhanced it, it is likely that CCK receptors other than types 1 or 2 are present in excitatory noncholinergic neural structures of the duodenal wall. Interestingly, a non-A non-B CCK receptor has been postulated to mediate the activation of primary gastric vagal afferents in OLETF rats that lack CCK-1 receptors (21).

Since the relaxation induced by CCK-8S and cerulein was also abolished by TTX we focused our experiments on the pharmacological characterization of the receptors and of the mediators involved in such effect. The fact that three selective CCK-1 receptor antagonists were able to reduce the magnitude of CCK-8S (and cerulein)-elicited relaxation in a concentration-dependent manner indicates that these responses are mediated predominantly by the activation of CCK-1 receptors. These receptors may be located either in nonadrenergic noncholinergic inhibitory neurons or in interstitial cells of Cajal since CCK-1 receptor immunoreactivity has been recently described in both cell types in the rat intestine (14,15). It is noteworthy that interstitial cells of Cajal have been postulated to mediate neurally induced relaxation in mice (22). The experimental design of the present study does not allow us to identify which of these cells is responsible for the observed effect. However, it is most likely that CCK-8S is acting directly on neurons since recent gene expression studies in the rat have shown that duodenal myenteric neurons express nuclear *c-fos* after *ip* CCK administration in a devazepide-sensitive manner (12). This interpretation is also consistent with the results of electrophysiological

Figure 3. Effect of 100 μ M L-NOARG on the magnitude of 300 nM CCK-8S-induced relaxation of rat duodenum longitudinal muscle in the absence and in the presence of L-arginine (L-ARG, 1 mM), or D-arginine (D-ARG, 1 mM). Data are reported as the means \pm SEM for 4-6 experiments. L-NOARG = N^onitro-L-arginine; CCK-8S = cholecystokinin-8S. *P < 0.05 compared to the control response (paired Student *t*-test).



studies in guinea pig myenteric neurons (11). Furthermore, the fact that L-NOARG was able to reduce the magnitude of the relaxation elicited by CCK-8S in an L-ARG-preventable manner indicates that they are mediated mainly by myenteric nitrenergic neurons. These neurons have been previously shown to be present in the myenteric plexus of the proximal rat duodenum and contribute to nonadrenergic noncholinergic nerve-mediated relaxation of this intestinal segment (16,23). This interpretation is consistent with

the recent observation that c-fos is induced in duodenal myenteric neurons expressing nitric oxide synthase after *ip* CCK administration to rats (13).

The present study showed that CCK-8S acting on CCK-1 receptors relaxes the longitudinal muscle of the rat proximal duodenum through the activation of intrinsic nitrenergic neurons. Whether these CCK-1 receptors are located exclusively on the neurons or in the interstitial cells of Cajal remains to be further investigated.

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