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CCK_2 receptors mediate inhibitory effects of cholecystokinin on the motor activity of guinea-pig distal colon

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Abstract

Cholecystokinin and related peptides are involved in the control of intestinal motility and cholecystokinin receptor ligands might represent new pharmacological tools for the treatment of symptoms associated with functional bowel disorders. However, the respective roles played by cholecystokinin receptor subtypes and the mechanisms underlying these regulatory actions remain undetermined. This study was designed to examine the influence of cholecystokinin receptor subtypes on the motor activity of guinea-pig distal colon. The effects of drugs acting on CCK_1 and CCK_2 receptors were assessed *in vitro* on the contractile activity of longitudinal smooth muscle, both under basal conditions and in the presence of transmural electrical stimulation or KCl-induced contractions. The application of cholecystokinin octapeptide sulphate (cholecystokinin-8S) to colonic preparations induced concentration-dependent contractions which were prevented by devazepide (CCK_1 receptor antagonist), enhanced by GV150013 (CCK_2 receptor antagonist) or N^{ω}-nitro-L-arginine methylester (L-NAME, nitric oxide synthase inhibitor), and unaffected by tetrodotoxin. The application of gastrin-17 to colonic preparations resulted in relaxant responses which were insensitive to devazepide, and prevented by GV150013, L-NAME or tetrodotoxin. L-NAME, N^{ω}-propyl-L-arginine (NPA, neuronal nitric oxide synthase inhibitor) or GV150013 enhanced electrically evoked contractile responses, whereas devazepide did not. When tested in the presence of L-NAME or NPA the enhancing effect of GV150013 on electrically induced contractions no longer occurred. In the presence of KCl-induced pre-contractions, cholecystokinin-8S or gastrin-17 evoked concentration-dependent relaxations, which were unaffected by devazepide and were counteracted by GV150013, L-NAME, NPA or tetrodotoxin. In conclusion, the present results indicate that, at level of distal colon, CCK_1 receptors mediate direct contractile effects on smooth muscle, whereas CCK_2 receptors on enteric neurons mediate relaxant responses via nitric oxide release.

Keywords: Cholecystokinin; Gastrin; Colonic motility; Nitric oxide; (Guinea-pig)

1. Introduction

Cholecystokinin, gastrin and related peptides include a family of peptide hormones and neuropeptides which exert a variety of physiological actions on the gastrointestinal tract, as well as on the central nervous system (Crawley and Corwin, 1994; Miyasaka and Funakoshi, 2003). Several studies, based on functional, pharmacological and molecular approaches, have indicated that the effects of these peptides are mediated by two different receptor subtypes designated as CCK_1 and CCK_2. CCK_1 receptors are mainly localized in the gastrointestinal tract and in few areas of the central nervous system, whereas CCK_2 receptors are widely expressed throughout the gastrointestinal tract and brain (Noble et al., 1999; Miyasaka and Funakoshi, 2003).

At intestinal level, CCK_1 receptors have been found both in myenteric neurons and longitudinal smooth muscle, and they have been implicated in the control of motor functions as well as in pain perception (Sternini et al., 1999; Varga et al., 2004). The role of these receptor subtypes in the regulation of colonic motility has been investigated both *in vitro* and *in vivo*, under physiological conditions and in the presence of inflammatory processes. Several lines of evidence have prompted the

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development of selective CCK_1 antagonists, currently under clinical investigation, which might represent new pharmacological tools for treatment of symptoms associated with functional bowel disorders (Varga et al., 2004; Cremonini et al., 2005). The prominent actions mediated by CCK_2 receptors within the digestive tract include stimulation of acid secretion from gastric parietal cells (Kulaksiz et al., 2000; Ochi et al., 2005) and release of histamine from enterochromaffin-like cells (Waldum et al., 2002), but they seem to contribute also to the regulation of gut motor functions (Giralt and Vergara, 1999; Dal Forno et al., 1992). The development of selective CCK_2 receptor antagonists, endowed with non-peptidic structure (Herranz, 2003), has fostered some interest for the role played by these receptors in the modulation of intestinal motility, although few data are available in this field (Vergara et al., 1996; Rodriguez-Membrilla and Vergara, 1997).

In different gut regions, the actions of cholecystokinin-related peptides on smooth muscle can be either direct or neurally mediated or both in nature. In general, it is thought that the direct effect results in contractile responses, whereas the neurally mediated actions can evoke either contractile or relaxant activity, depending on the nature of transmitter being released (Varga et al., 2004). However, the mechanisms underlying the control of intestinal motor activity by CCK_1 and CCK_2 receptors are only partly understood, and several aspects remain to be clarified. This was the basis for the present study to investigate the effects of drugs acting at cholecystokinin receptor subtypes on both spontaneous and stimulated colonic motility under *in vitro* conditions. A major purpose was the characterization of mechanisms underlying the modulating effects of CCK_2 receptors on colonic motor activity.

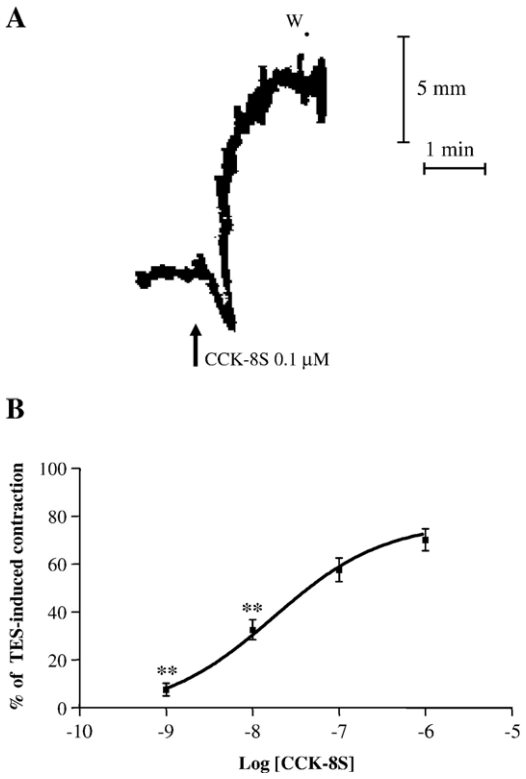

Fig. 1. (A) Representative trace recording showing the effect of cholecystokinin-8S (0.1 μM) on the motor activity of distal colonic preparations. (B) Effects of increasing concentrations of cholecystokinin-8S on the contractile activity of longitudinal muscle. Each point represents the mean of eight experiments ± S.E.M. (vertical bars). Analysis of data in panel B: ANOVA for repeated measures (df=31, F=52.032, P<0.0001); Dunnett's test: **P<0.01, significant difference vs. maximal contraction induced by cholecystokinin-8S at the concentration of 1 μM. W, washing; CCK-8S, cholecystokinin-8S.

2. Methods

2.1. Animals

Albino male guinea-pigs (Harlan Italy, Udine, Italy), 300–350 g body weight, were used throughout the study. They were housed in temperature-controlled rooms, in a 12-h light–dark cycle (starting at 7:30 a.m.) at 22–24 °C and 50–60% humidity, and were not employed for at least 1 week after their delivery to the laboratory. Their care and handling were in accordance with the provision of the European Union Council Directive 86/609, recognized and adopted by the Italian Government.

2.2. Recording of longitudinal muscle contractile activity

The contractile activity of colonic longitudinal smooth muscle was recorded as previously described by Blandizzi et al. (2003). At the time of sacrifice (9:00 a.m.), the whole colon was excised and placed into cold pre-oxygenated Krebs solution. Segments of distal colon, approximately 20 mm in length, were then prepared. The preparations were set up in 10-ml organ baths containing Krebs solution at 37 °C, bubbled with 95% O_2 + 5% CO_2, connected vertically to isotonic transducers (Basile, Comerio, Italy) under a constant load of 1 g, and allowed to equilibrate for 30 min. Krebs solution was as follows (mM): NaCl 113, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 11.5 (pH 7.4 ± 0.1). The contractile activity was recorded by a polygraph (Basile, Comerio, Italy). A pair of coaxial platinum electrodes was positioned at distance of 10 mm on the longitudinal axis of each preparation to deliver transmural electrical stimulation by a BM-ST6 stimulator (Biomedica Mangoni, Pisa, Italy). Stimuli were applied as 20-s single trains of square wave pulses (1 ms, 30 mA, 10 Hz). Each preparation was repeatedly challenged with electrical stimulations, and experiments started when reproducible responses were obtained (usually after 2–3 stimulations).

The first set of experiments was performed to evaluate the effects of cholecystokinin octapeptide sulphate (cholecystokinin-8S) and gastrin-17 on spontaneous motor activity of colonic preparations. For this purpose, non-cumulative concentration-response curves were constructed for cholecystokinin-8S (0.001–1 μM) and gastrin-17 (0.001–1 μM) on basal contractility of longitudinal muscle. In the second set, the effects of cholecystokinin-8S (0.1 μM) and gastrin-17 (0.1 μM) were examined on spontaneous colonic activity after incubation with increasing concentrations of the selective CCK_1 and CCK_2 receptor antagonists devazepide (0.001–1 μM) and GV150013 (0.001–1 μM), respectively.