Fundam Clin Pharmacol 1997;11:231–236

Original article

Involvement of a CCK-dependent capsaicin-sensitive afferent pathway in the inhibitory effect of pinaverium bromide on the colonic motor response to eating in rats

J Fioramonti[1], MO Christen[2], I Dupre[1], L Bueno[1]

[1] *Department of Pharmacology, Institut National de la Recherche Agronomique, 180 Chemin de Tournefeuille, BP 3, 31931 Toulouse cedex 9;* [2] *Solvay Pharma France, Suresnes, France*

(Received 18 October 1996; revised 20 January 1997; accepted 28 January 1997)

Summary – The effects of pinaverium bromide on the stimulation of colonic motility induced by meal and cholecystokinin (CCK) were investigated in rats chronically fitted with intraparietal electrodes on the proximal colon and previously treated or not by capsaicin. Pinaverium bromide inhibited in a dose-related manner (2–50 mg/kg, per os) the increase in colonic spike burst frequency induced by a 3 g meal or CCK-8 (2 μg/kg, iv). The CCK-A and CCK-B antagonists, devazepide and L 365260 (100 μg/kg, ip), respectively, inhibited the postprandial colonic motor response while only L 365260 reduced the CCK-induced stimulation. The effects of pinaverium bromide and CCK antagonists were not observed in capsaicin-treated animals. Moreover, CCK-8 (2 μg/kg, iv) did not stimulate colonic motility after capsaicin treatment. The inhibition of postprandial colonic motility by pinaverium bromide, given orally at therapeutic doses, involves a CCK-dependent pathway which requires the integrity of capsaicin-sensitive afferents.

pinaverium / colonic motility / CCK antagonists / capsaicin / gastrointestinal afferents

INTRODUCTION

Pinaverium bromide, a quaternary ammonium compound, has been recommended for the treatment of intestinal motor disorders in irritable bowel syndrome (Christen, 1990). Among the mechanisms proposed to explain its efficacy is an inhibition of the postprandial propulsive colonic contractions (Fioramonti et al, 1988). It is considered a smooth muscle relaxant since it inhibits contractions of isolated intestine induced by barium chloride, field stimulation or acetylcholine (Baumgartner et al, 1985; Droogmans et al, 1983). It has been clearly shown that pinaverium bromide is a calcium antagonist, able to block voltage-dependent L-type Ca^{2+} channels and to bind to the α-1 subunit of the Ca^{++} channel in intestinal smooth muscle cells (Beech et al, 1990; Feron et al, 1992).

It has also been shown that pinaverium bromide inhibits the contraction of isolated intestinal smooth muscle cells induced by cholecystokinin (CCK) (Bobo et al, 1994). This action very likely involves the calcium antagonist properties of the compound which displayed a potency equivalent to that of diltiazem (Bobo et al, 1994). On the other hand CCK has been involved in the colonic motor response to a meal in humans and animals. In humans, CCK administration reproduces the postprandial pattern of colonic myoelectric activity (Renny et al, 1983) and in rats, central or peripheral administration of CCK antagonists attenuates the postprandial increase in the frequency of colonic spike bursts (Liberge et al, 1991; Fioramonti et al, 1994). CCK receptors have been found localized at the periphery and in the central nervous system, but those localized on afferent neurons (Mercer et al, 1992) are of paramount importance in mediation of the effects of CCK. For example, the inhibition of gastric emptying induced by CCK has been found to be mediated through a vagal afferent pathway (Raybould and Taché, 1988).

Consequently, this study aimed to determine whether pinaverium bromide inhibits the colonic

motor response to eating in rats via a mechanism involving CCK receptors and afferent neurons. The role of afferents was studied using the neurotoxin capsaicin which selectively induces a degeneration of primary afferent neurons (Holzer, 1991).

MATERIALS AND METHODS

Animal preparation

Four groups of six male Wistar rats weighing 250–300 g were used in these experiments. Animals were individually housed in polypropylene cages and kept in a temperature-controlled room (21 ± 1 °C). They were allowed free access to water and were fed laboratory pellets (UAR, Epinay, France). Each animal was prepared for long-term recordings of cecocolonic myoelectric activity using a previously described technique (Ruckebusch and Fioramonti, 1975). Briefly, under ketamine anesthesia, nichrome wire electrodes (80 μm in diameter and 80 cm in length) were implanted in the wall of the proximal colon (2 cm from the caecum). Electrodes were exteriorized on the back of the neck and protected by a glass tube attached to the skin.

Two weeks before surgery for electrode implantation, two groups of animals (groups two and four) were treated by systemically applied capsaicin, which was injected subcutaneously on four consecutive days at a rate of two injections per day with increasing doses to reach a total dose of 100 mg/kg. The effectiveness of capsaicin treatment was tested by means of the eye-wiping test, which consists of impaired chemosensitivity of corneal afferents to one drop of 1% NH4OH instilled into the eye. This impairment consisted of either the absence of any movement, or solely uncompleted paw movements which did not reach the eyes.

Myoelectric recordings

Electromyographic recordings were started 5 days after surgery. Spiking activity was recorded with an electroencephalograph (Mini VIII, Alvar, Paris, France) using a short time constant (0.03 s) to selectively record spike bursts at a paper speed of 3.6 cm/min. The frequency of colonic spike bursts was expressed in number of bursts occurring in 10 min periods.

Experimental design

The experiments were performed in rats fasted for 12 hours but with free access to water. Animals of groups one and two received 3 g of lab chow meal preceded 1 hour before by an orogastric administration of pinaverium bromide (1–50 mg/kg), or 10 min before the meal by an ip administration of devazepide (100 μg/kg) or L 365260 (100 μg/kg). The animals were previously accustomed to eating a 3 g meal after 12 hours of fasting. Groups three and four received an iv administration of CCK-8 (2 μg/kg) preceded by pinaverium bromide or CCK antagonists, given in the same conditions as before the meal in groups one and three. Controls consisted of oral administration of water and ip injection of saline. Oral administrations were performed in a 0.5 mL volume, and iv and ip in 0.2 mL.

Pinaverium bromide was provided by Solvay Pharma France, CCK-8 and capsaicin were purchased from Sigma Chemical Co (St Louis, MO, USA). Devazepide and L 365260 were gifts from Merck Institute for Therapeutic Research (West Point, PA, USA).

Statistical analysis

Values were means ± SD. They were compared using analysis of variance (ANOVA) and Student's t-test for unpaired values. Statistical significance was accepted if $P < 0.05$.

RESULTS

Intact animals

In the fasted state (during the 30 min period preceding the meal) the frequency of long spike bursts (LSB) occurring on the proximal colon was 9.4 ± 1.5 per 10 min ($n = 6$). Ingestion of a 3 g meal significantly increased ($P < 0.05$) the spike burst frequency starting from 30 min after the beginning of the meal, with a maximum of 21.5 ± 1.2 LSB/10 min ($n = 6$) being observed during the first 10 min at the colonic level (fig 1, 2). Pinaverium bromide, given orally 1 h before the meal in the dose range of two to 50 mg/kg did not significantly modify ($P > 0.05$) the frequency of colonic LSB in the fasted state but, in a dose-related manner, significantly reduced ($P < 0.05$) the postprandial increase in LSB frequency (fig 3). Devazepide and L 365260 (CCK-A and CCK-B antagonists, respectively) given ip 10 min before the meal also significantly reduced ($P < 0.05$) by 35 and 31%, respectively, the postprandial increase in LSB frequency (table I).

CCK-8, administered iv at a dose of 2 μg/kg, induced a significant ($P < 0.05$) increase of 3.9 ± 1.0 LSB during the first 10 min. Pinaverium bromide (2–50 mg/kg), given orally 1 h before CCK-8 injection, significantly inhibited ($P < 0.05$) this stimulation, with a maximal effect being reached at the 5 mg/kg dose (fig 4). The CCK-A

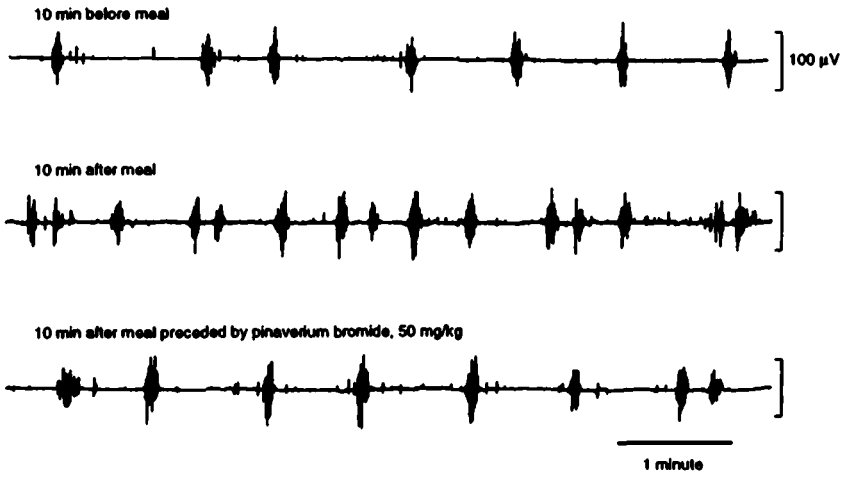

Fig 1. Colonic electromyograms showing that the postprandial increase in spike burst frequency was reduced when pinaverium bromide was administered orally, at a dose of 50 mg/kg, one hour before the meal.

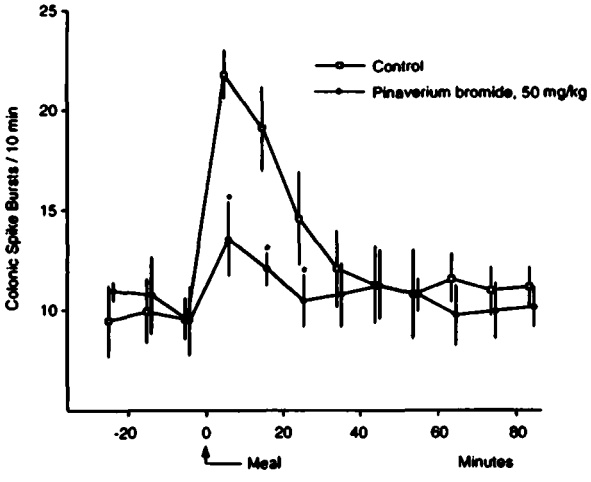

Fig 2. Postprandial evolution of colonic spike burst frequency in control studies and after pinaverium bromide, given orally one hour before the meal. Values are means ± SD (n = 6). * $P < 0.05$ from control values.

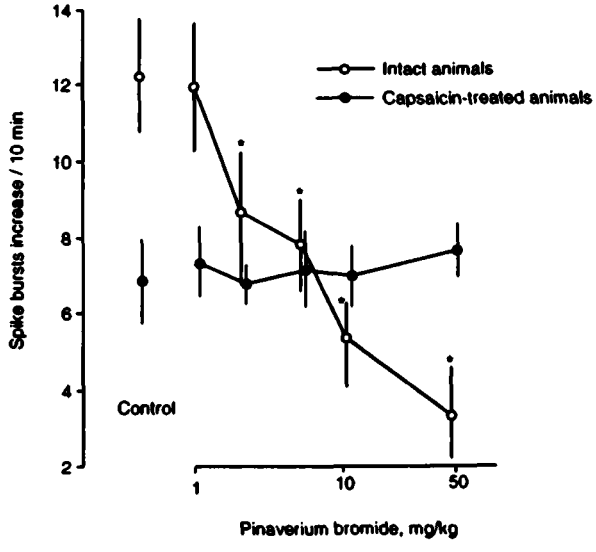

Fig 3. Effects of increasing doses of pinaverium bromide given orally, one hour before the meal, on the increase in colonic spike burst frequency observed during the first 10 min after a 3 g meal, in intact and capsaicin-treated animals. Values are means ± SD (n = 6). * $P < 0.05$ from control values.

antagonist devazepide (100 µg/kg, ip) did not modify the CCK-induced stimulation that the CCK-B antagonist, L 365260 at the 100 µg/kg ip dose significantly reduced ($P < 0.05$) by 72% (table I).

Capsaicin-treated animals

In the fasted state, the frequency of colonic LSB was significantly ($P < 0.05$) higher than that observed in intact animals (12.5 ± 1.7/10 min vs 9.4 ± 1.5, n = 6). The maximal LSB frequency observed during the first 10 min following the 3 g meal was significantly ($P < 0.05$) lower than in intact animals (19.0 ± 2.0/10 min vs 21.5 ± 1.2, n = 6). Consequently, the amplitude of the postprandial increase in LSB frequency was in the cap-

Table I. Effects of CCK antagonists on the increase in spike burst frequency (number/10 min) observed during the first 10 minutes following meal ingestion and CCK-8 administration in intact and capsaicin-treated animals.

Treatment	*Intact animals*		*Capsaicin-treated animals*	
	Meal 3 g	*CCK-8* 2 μg/kg, iv	*Meal* 3 g	*CCK-8* 2 μg/kg, iv
Saline	12.1 ± 0.9	3.9 ± 1.0	6.7 ± 0.6†	0.2 ± 0.9†
Devazepide 100 μg/kg, ip	7.9 ± 1.0*	4.1 ± 0.7	7.4 ± 0.9	ND
L 365260 100 μg/kg, ip	8.4 ± 0.7*	1.1 ± 0.6*	6.4 ± 0.7	ND

Values are means ± SD (n = 6). * $P < 0.05$ vs saline; † $P < 0.05$ vs intact animals; ND: not determined.

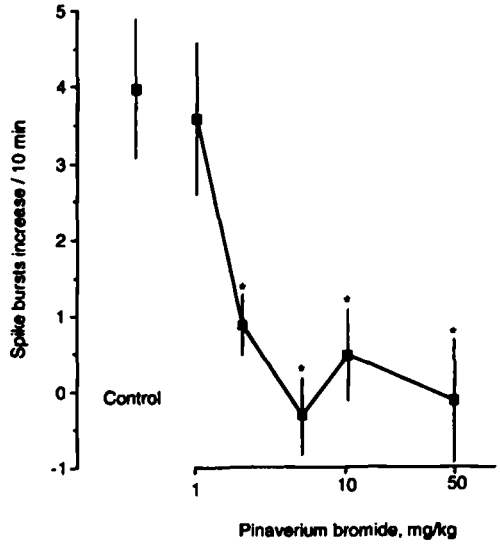

Fig 4. Effects of pinaverium bromide on the increase in colonic spike burst frequency observed during the first 10 min after CCK-8 (2 μg/kg, iv). Pinaverium bromide was given orally 1 hour before CCK-8. Values are means ± SD (n = 6). * $P < 0.05$ from control values.

saicin-treated animals nearly half than that observed in control animals (fig 3).

Pinaverium bromide, whatever the dose from one to 50 mg/kg, did not significantly modify ($P > 0.05$) the postprandial increase in LSB frequency in capsaicin-treated animals (fig 3). Similarly, neither devazepide nor L 365260 significantly modify ($P > 0.05$) this postprandial stimulation (table I).

Moreover, CCK-8 administered iv (2 μg/kg) in capsaicin-treated animals did not induce any significant ($P > 0.05$) change in the frequency of colonic LSB (table I).

DISCUSSION

Our data indicate that the calcium channel blocker, pinaverium bromide, reduces the colonic motor response to a meal and to CCK. These effects were reproduced by CCK-A and/or CCK-B antagonists and require the integrity of capsaicin-sensitive afferents.

The inhibition of the colonic motor response to the meal was dose-related from a very low dose of 2 mg/kg and was obtained with therapeutic doses of pinaverium bromide, currently used in the treatment of irritable bowel syndrome (Galeone et al, 1986). Disturbances of the colonic motor response to eating is a characteristic of irritable bowel syndrome (Narducci et al, 1986) and this response has been found to be inhibited by other calcium antagonists such as nifedipine (Narducci et al, 1985) or octylonium bromide (Narducci et al, 1986). Moreover, the antagonistic action of pinaverium bromide against the stimulation of colonic motility by CCK is in agreement with the efficacy of pinaverium bromide in the treatment of irritable bowel syndrome because CCK-8, infused iv, has been found to induce pain and nausea in patients with functional abdominal pain symptoms, but not in control subjects (Roberts-Thomson et al, 1992).

The inhibition of the colonic motor response to CCK by pinaverium bromide confirms *in vivo* the inhibition of isolated smooth muscle cell contraction induced by CCK, a calcium-dependent phenomenon, and is in agreement with the calcium antagonist properties of pinaverium bromide because other calcium antagonists such as diltiazem and nicardipine are also able to inhibit the CCK-induced contraction of smooth muscle cells (Bobo et al, 1994). The reduction by pinaverium bromide of the stimulation of colonic motility by both meal and CCK indicate that a CCK pathway is involved in the action of pinaverium bromide since the colonic motor response to food has been found to be mediated in rats through CCK receptors. At the peripheral level, both CCK-A and CCK-B receptors have been found to be involved in the postprandial increase in colonic motility (Fioramonti et al, 1994), and this was confirmed in the present study. At the hypothalamic level, CCK-A receptors have been found to be involved in this response (Liberge et al, 1991). However, in this study, the increase in colonic motility induced by the meal and CCK-8 was reduced by a CCK-B antagonist, while a CCK-A antagonist reduced

only the response to the meal. This result indicates that CCK-8, which is one of the numerous molecular forms of endogenous CCK, does not fully reproduce the stimulation of colonic motility induced by the meal. Moreover, our results do not indicate that pinaverium bromide acts selectively through a CCK-dependent pathway, and an action through other pathways cannot be excluded.

The most striking result of the present study is that the effects of pinaverium bromide were abolished in capsaicin-treated animals. This is in agreement with the CCK-mediated action of pinaverium bromide. Capsaicin is known to have a neurotoxic action on afferent neurons with unmyelinated axons (C-fibers) or with thinly myelinated axons (Aδ-fibers) (Raybould and Taché, 1988). Tachykinins, like substance P and calcitonin gene-related peptide, are the major neurotransmitters released from capsaicin-sensitive afferents. However, CCK immunoreactivity and CCK binding sites have been identified on these neurons (Gibbins et al, 1987; Ladenheim et al, 1986).

Moreover, several effects of CCK administered peripherally have been found to be mediated through capsaicin-sensitive afferent fibres. These effects are as different as inhibition of gastric emptying, suppression of food intake (Ritter and Ladenheim, 1985), protection against ethanol-induced gastric lesions (Evangelista and Maggi, 1991), or stimulation of neurohypophyseal secretion of oxytocin (McCann et al, 1988). Our data indicate that the colonic motor response to CCK is also mediated through capsaicin-sensitive afferents. However, most of these effects are very likely mediated through capsaicin-sensitive vagal afferents, thus agreeing with the presence of CCK binding sites in the cervical and subdiaphragmatic vagus nerve as well as in the nucleus of the solitary tract and area postrema – sites of termination for vagal afferent fibres (Moran et al, 1990).

The absence of effect of CCK antagonists on the postprandial increase in colonic motility in capsaicin-treated animals is in agreement with recent data obtained at the small intestine level (Rodriguez-Membrilla and Vergara, 1995). The authors showed that after suppression of vagal afferents by capsaicin, the postprandial changes in small intestine pattern persisted, but were not reduced, by CCK antagonists, as they were in intact animals. Our data cannot permit to indicate that the inhibition of the colonic motor response to eating either by CCK antagonists or pinaverium bromide involves vagal afferents as capsaicin has been administered systemically and was not locally applied on the vagus nerve, and since capsaicin-sensitive fibres have been identified in splanchnic nerves (Maggi, 1990). Nevertheless, we can postulate that pinaverium bromide and CCK antagonists implicate the vagal afferent pathway involved in the vago-vagal gastro-colonic reflex triggered by gastric distension, in turn induced by meal ingestion and that activation of vagal afferents involves CCK receptors.

In conclusion, since two main characteristics of irritable bowel syndrome are disturbances of the colonic motor response to eating (Narducci et al, 1986) and hypersensitivity to gut stimuli (Accarino et al, 1995), the inhibitory action of pinaverium bromide of postprandial motility through a mechanism involving sensory afferent neurons could explain the efficacy of this compound in irritable bowel syndrome by acting both on motility and hypersensitvity of the gut.

ACKNOWLEDGEMENT

This study was supported by a grant from Solvay Pharma France.

REFERENCES

Accarino AM, Azpiroz F, Malagelada JR. Selective dysfunction of mechanosensitive intestinal afferents in irritable bowel syndrome. *Gastroenterology* 1995;108:636–43

Baumgartner A, Drack E, Halter F, Scheurer U. Effects of pinaverium bromide and verapamil on the motility of the rat isolated colon. *Br J Pharmacol* 1985;86:89–94

Beech DJ, MacKenzie I, Bolton TB, Christen MO. Effects of pinaverium on voltage-activated calcium channel currents of single smooth muscle cells isolated from the longitudinal muscle of the rabbit jejunum. *Br J Pharmacol* 1990;99:373–8

Bobo MH, Magous R, Christen MO, Bali JP. Effect of pinaverium and other calcium channel blockers on contraction of isolated gastric antral smooth muscle cells caused by gastrointestinal hormones. *Life Sci* 1994;54:1947–54

Christen MO. Action of pinaverium bromide, a calcium–antagonist, on gastrointestinal motility disorders. *Gen Pharmacol* 1990;21:821–5

Droogmans G, Himpens B, Casteels R. Effect of pinaverium bromide on electrical and mechanical activity of smooth muscle cells. *Naunyn-Schmiedeberg's Arch Pharmacol* 1983;323:72–7

Evangelista S, Maggi CA. Protection induced by cholecystokinin-8 (CCK-8) in ethanol-induced gastric lesions is mediated via vagal capsaicin-sensitive fibres and CCKA receptors. *Br J Pharmacol* 1991;102:119–22

Feron O, Wibo M, Christen MO, Godfraind T. Interaction of pinaverium (a quaternary ammonium compound) with 1,4-dihydropyridine binding sites in rat ileum smooth muscle. *Br J Pharmacol* 1992;105:480–4

Fioramonti J, Fargeas MJ, Bertrand V et al. Induction of postprandial motility and release of cholecystokinin by polyamines in rats. *Am J Physiol* 1994;267:G960–5

Fioramonti J, Frexinos J, Staumont G, Buéno L. Inhibition of the colonic motor response to eating by pinaverium bro-

mide in irritable bowel syndrome patients. *Fundam Clin Pharmacol* 1988;2:19–27

Galeone M, Stock F, Moise G et al. Pinaverium bromide versus otilonium bromide in patients with irritable bowel syndrome. *Curr Ther Res* 1986;39:613–24

Gibbins IL, Furness JB, Costa M. Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurons of the dorsal root ganglia of the guinea-pig. *Cell Tissue Res* 1987;248:417–37

Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. *Pharmacol Rev* 1991;43:143–201

Ladenheim EE, Speth RC, Ritter RC. Reduction of CCK-8 binding in the nucleus of the solitary tract of capsaicin pretreated rats. *Soc Neurosci Abstr* 1986;12:828

Liberge M, Arruebo MP, Buéno L. Role of hypothalamic cholecystokinin octapeptide in the colonic motor response to a meal in rats. *Gastroenterology* 1991;100:441–9

Maggi CA. Capsaicin-sensitive nerves in the gastrointestinal tract. *Arch Int Pharmacodyn Ther* 1990;303:157–66

McCann MJ, Verbalis JG, Stricker EM. Capsaicin pretreatment attenuates multiple responses to cholecystokinin in rats. *J Auton Nerv Syst* 1988;23:265–72

Mercer JG, Farningham DAH, Lawrence CB. Effect of neonatal capsaicin treatment on cholecystokinin-(CCK-8) satiety and axonal transport of CCK binding sites in the rat vagus nerve. *Brain Res* 1992;569:311–6

Moran TH, Norgren R, Crosby RJ, McHugh PR. Central and peripheral vagal transport of cholecystokinin binding sites occurs in afferent fibres. *Brain Res* 1990;526:95–102

Narducci F, Bassotti G, Gaburri M et al. Nifedifine reduces the colonic motor response to eating in patients with the irritable bowel syndrome. *Am J Gastroenterol* 1985;80:317–9

Narducci F, Bassotti G, Granata MT et al. Colonic motility and gastric emptying in patients with irritable bowel syndrome. Effect of pretreatment with octylonium bromide. *Dig Dis Sci* 1986;31:241–6

Raybould HE, Taché Y. Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. *Am J Physiol* 1988;255:G242–6

Renny A, Snape WJ, Sun EA et al. Role of cholecystokinin in the gastrocolonic response to a fat meal. *Gastroenterology* 1983;85:17–21

Ritter RC, Ladenheim EE. Capsaicin pretreatment attenuates suppression of food intake by cholecystokinin. *Am J Physiol* 1985;248:R501–4

Roberts-Thomson IC, Fettman MJ, Jonsson JR, Frewin DB. Responses to cholecystokinin octapeptide in patients with functional abdominal pain syndromes. *J Gastroenterol Hepatol* 1992;7:293–7

Rodriguez-Membrilla A, Vergara P. Meal activates both CCK-dependent vagal fibers and CCK-independent pathway to regulate intestinal motility. *Neurogastroenterol Mot* 1995;7:283

Ruckebusch M, Fioramonti J. Electrical spiking activity and propulsion in small intestine in fed and fasted rats. *Gastroenterology* 1975;68:1500–8