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Exogenous cholecystokinin-8 reduces vagal efferent nerve activity in rats through CCK_A receptors

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1 It has been proposed that the vagus nerve plays a role in mediating cholecystokinin-8 (CCK-8) effect on such gastric functions as motility, emptying and gastric acid secretion. To examine the contribution of the efferent pathways in realizing these effects, efferent mass activity in the ventral gastric vagal nerve in Sprague-Dawley rats was recorded.

2 Intravenous infusion of CCK-8 (0.1–1 nmol) suppressed the efferent activity. The effect of CCK-8 was significantly reduced in animals with total subdiaphragmatic vagotomy in comparison to those with partial vagotomy.

3 Intravenous infusion of CCK_A receptor antagonist L-364,718 ($1-100\times10^{-6}$ g) blocked the response of vagal efferent activity to 0.1 nmol CCK-8, but the CCK_B receptor antagonist L-365,260 ($1-100\times10^{-6}$ g) did not in the conditions of either partial or total vagotomy.

4 Intracisternal infusion of L-364,718 (1×10^{-6} g) blocked the response of vagal efferent activity to 0.1 nmol CCK-8 i.v.

5 Infusion of exogenous CCK-8 did not affect the activity of supradiaphragmatic vagal afferents.

6 The results suggest that the effect of systemically administered CCK-8 on vagal efferent activity is mediated by both peripherally (subdiaphragmatically) and centrally localized CCK_A receptors.
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Keywords: Cholecystokinin-8; CCK-8 receptors; CCK_A; CCK_B; vagus; visceral efferents

Abbreviations: CCK_A, type A CCK receptor; CCK_B, type B CCK receptor; CCK-8, cholecystokinin-8 (26–33); DMX, dorsal motor nucleus of the vagus nerve; NTS, nucleus tractus solitarius; L-364,718, type A CCK antagonist; L-365,260, type B CCK antagonist

Introduction

Neuropeptide cholecystokinin-8 (CCK-8) which is released into the circulation during feeding (Lewis & Williams, 1990) has been shown to excite vagal gastric, hepatic as well as intestinal afferents (Cox & Randich, 1997; Kurosawa *et al*., 1997; Richards *et al*., 1996; Schwartz *et al*., 1994). Excitation of the vagal afferent nerve conveys satiation signals to the brain (Mayne *et al*., 1998), resulting in the inhibition of the food intake. At the same time the vagal afferent excitation in response to CCK-8 contributes to the reflex regulation of pancreatic secretion (Li *et al*., 1997), gastric motility and emptying (Raybould & Taché, 1988) as well as gastric acid secretion (Raybould & Lloyd, 1994). The vagal efferent nerve is suggested to be one of the major efferent limbs of this reflex regulation. However, there has been no study addressing the responses of the vagal efferent activity in response to CCK-8.

The effect of CCK-8 is mediated *via* CCK_A and CCK_B receptors. In general, CCK_A receptors are abundant in the gastrointestinal tract while CCK_B receptors are found dominantly in the brain. However, both CCK_A and CCK_B receptors are described on the vagal nerves (Mercer & Lawrence, 1992), the nucleus tractus solitarius (NTS) where vagal afferents terminate, and the dorsal motor nucleus of the vagal nerve (DMX) where vagal efferents emerge (Plata-Salamán *et al*., 1988). Although it has been shown that the gastric vagal afferent fibres are activated *via* the CCK_A receptors in response to exogenously administered CCK-8 (Kurosawa *et al*., 1997; Schwartz *et al*., 1994), the response of the gastric vagal efferents might include the activation of both CCK_A and CCK_B receptors at brain stem level, including DMX and NTS (Branchereau *et al*., 1993).

In the present study the response of gastric vagal efferent fibres to exogenously administered CCK-8 and the mechanisms involved have been investigated. First, the activity of vagal efferents was recorded under conditions of partial or total subdiaphragmatic vagotomy to elucidate the extent of the contribution of subdiaphragmatic vagal afferents to the response. Second, the site of action of CCK-8 and its receptor mechanisms were investigated in animals with partial or total subdiaphragmatic vagotomy in order to explore mechanisms additional to those of the vagal afferent-mediated. Finally, the response of supradiaphragmatic vagal afferent activity to intravenous CCK-8 was investigated.

Methods

Animals

Male Sprague-Dawley rats (ALAB Sollentuna, Sweden) 350 ± 70 g, were housed five per cage at 21°C, with water and food *ad libitum* and a 12 h light/dark cycle. Animals were deprived of food for 14–20 h before the experiment. Experiments were approved by the Karolinska Institutet local animal ethical committee.

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Experimental procedures

Rats were anaesthetized with pentobarbital sodium (60 mg kg^{-1}, i.p.). Artificial ventilation was carried out throughout the experiments *via* a tracheal cannula (Harvard ventilator, model 683). Body temperature was maintained at $+37.2\pm0.1$°C (ATB-1100, Nihon Kohden, Japan). Blood pressure was continuously monitored from the femoral artery and maintained above 90 mmHg (systolic) by administration of 4% Ficoll 70. Additional pentobarbital sodium (5 mg h^{-1} kg^{-1}) and muscle relaxant, gallamine triethiodide (20 mg h^{-1} kg^{-1}) were injected through the right jugular vein using a syringe pump (model STC-521, Terumo, Tokyo, Japan). Experimental substances were injected through the right femoral vein (i.v.) or into the cisterna magna (i.c.m.). For the i.c.m. injections, the dura mater covering the foramen magnum was exposed. A small hole was made with a 25 gauge needle at the midline below the caudal edge of the occipital bone and 0.4–0.6 cm of polyethylene tube (PE 10, I.D. 0.28 mm, O.D. 0.61 mm; dead space 5 μl) filled with saline was inserted. To ensure the stable position of the catheter, a drop of Swebond (acrylic dental material, Svedia Dental-Industri AB, Sweden) was used. Free-leaking of clear CSF was considered as being proof of successful catheterization. The catheter was connected to an Exmire microsyringe (10 μl) using a silicone tube.

The abdomen was opened by midline section and the gastric branch of the ventral subdiaphragmatic vagal nerve was identified on the oesophagus, separated from the surrounding tissues under a binocular microscope and cut just proximal from the entrance into the stomach. The dorsal subdiaphragmatic vagus was left intact ($n=8$) or cut beneath the diaphragm ($n=8$). All branches of the ventral subdiaphragmatic vagal nerve including the hepatic, intestinal and accessory coeliac branches were cut in all animals (Figure 1). The central cut segment of the ventral gastric branch was placed on a bipolar platinum wire electrode and immersed in paraffin oil. The mass efferent activity was amplified (amplification gain 10 μV, bandpass 150–1000 Hz, time constant 0.01 s) (MEG-1200, Nihon-Kohden, Japan), counted every 2 s by a pulse counter after passing through a window discriminator (MET-1100, Nihon-Kohden, Japan) and recorded on a polygraph (WS-682G, Nihon-Kohden, Japan). The lower level of the window discriminator was always set clearly above the maximal noise peak level.

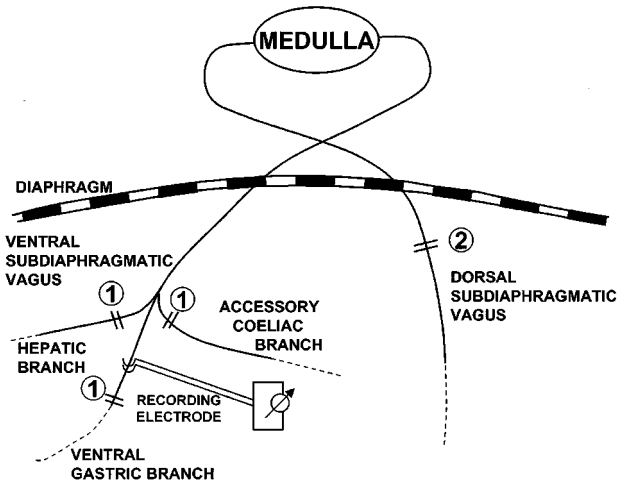

Figure 1 Schematic view of the two types of subdiaphragmatic vagotomies used in the present experiments. Partial subdiaphragmatic vagotomy consisted of complete transsection of hepatic, ventral gastric and accessory coeliac branches (marked 1). Total subdiaphragmatic vagotomy additionally transsected dorsal subdiaphragmatic vagus (marked 2). Electrical activity was recorded from the proximal segment of the ventral gastric branch.

The supradiaphragmatic afferent activity was recorded from the distal cut segment of a filament dissected from a bundle of the left cervical vagal nerve ($n=8$) in animals with total subdiaphragmatic vagotomy.

Sulphated cholecystokinin octapeptide (CCK-8) were first dissolved in saline containing 0.1% bovine serum albumine (BSA) and diluted with saline. Then it was administered intravenously as bolus injections in doses of 0.01, 0.1 or 1 nmol (ca 0.35–3.5 μg kg^{-1}) in 0.2 ml saline given over 20 s. L-364,718, a CCK_A receptor antagonist, and L-365,260, a CCK_B receptor antagonist, were dissolved in 50% dimethyl sulfoxide (DMSO) at a concentration of 12.5 μmol ml^{-1}, and then diluted with saline. Antagonists were injected intravenously as bolus injections in doses of 1, 10 and 100 μg in 0.2 ml saline 5 min before the administration of CCK-8 (0.1 nmol) ($n=8$). In six rats 1 μg L-364,718 in 6 μl saline was injected i.c.m. and CCK-8 (0.1 nmol) subsequently injected i.v. every 10 min over a 1 h period.

Drugs

The following drugs were used: pentobarbital sodium (Apoteksbolaget, Sweden); Ficoll 70 (Pharmacia Fine Chemicals AB, Uppsala, Sweden); gallamine triethiodide, cholecystokinin-8 (CCK 26–33), dimethyl sulfoxide (DMSO) and bovine serum albumine (BSA) from Sigma Chemical Co., St. Louis, MO, U.S.A.; paraffin oil (Kebo Lab.); L-364,718 (devazepide, or MK-329) and L-365,260 from ML Lab, London, U.K.

Statistical analysis

Responses of vagal gastric efferent activity were measured over 10 s immediately before the onset of CCK-8 administration and expressed as 100% control value. This was then compared with the average count over 10 s in every 30 s (start of the injection taken as time point zero), expressed as mean$\pm$ s.e.mean. Data were analysed by two-way repeated-measures ANOVA. When a significant treatment effect was found, Dunnett's test for each group and time point was performed. Comparisons of latencies of the response were made by Student's *t*-test, two-tailed. Probability less than 5% was considered significant.

Results

Effect of exogenous i.v. CCK-8 on vagal efferent activity in animals with partial subdiaphragmatic vagotomy

The efferent activity of the gastric vagal nerve was recorded from a branch of the central cut segment of the sectioned whole ventral subdiaphragmatic vagal nerve, i.e. the ipsilateral subdiaphragmatic vagal afferent activity was disrupted.

As shown in Figure 2, 0.1 and 1 nmol doses of CCK-8 produced a rapid, significant dose-dependent decrease in the mass activity of the gastric vagal efferent nerve (treatment $\times$ time interaction $F(3, 33)=11.2$, $P<0.001$). Administration of 0.1 nmol of CCK-8 decreased the nerve activity to 33 ± 5% and 52 ± 6% of the pre-administration control at 30 and 60 s, respectively. The activity returned to its basal level 2.5 min after the administration. Injection of 1.0 nmol CCK-8 further decreased the nerve activity to 28 ± 6% and 27 ± 6% of pre-

administration control at 30 and 60 s after the administration, respectively. This decrease lasted for more than 5 min after the injection. After 1.0 nmol CCK-8 the mean latency of the response was 12±0.6 s and after 1 nmol CCK-8 10.5±0.44 s. Intravenous administration of saline or a lower dose of CCK-8 (0.01 nmol) had no effect on the mass activity of the gastric vagal efferent nerve. In other experiments, BSA or DMSO alone were also without effect on gastric vagal efferent nerve activity (data not shown).

Effect of exogenous i.v. CCK-8 on vagal efferent activity in animals with partial subdiaphragmatic vagotomy after i.v. pretreatment with either CCK_A or CCK_B receptor antagonist

To study the contribution of CCK_A and CCK_B receptors to the response of vagal efferent activity in rats with partial subdiaphragmatic vagotomy, CCK_A or CCK_B receptor antagonist was administered before the injection of 0.1 nmol CCK-8. Neither the CCK_A nor CCK_B receptor antagonist itself affected the spontaneous efferent activity of the vagal nerve (data not shown).

Intravenous injection of L-364,718, a CCK_A receptor antagonist in dose of 1 μg, 5 min prior to the injection of 0.1 nmol CCK-8 partially blocked the response of vagal efferent activity in comparison to that of CCK-8 injected after saline (Figure 3A). Larger doses of the antagonist (10 and 100 μg) completely blocked the response (treatment × time interaction $F(3,33)=11.5$, $P<0.001$).

Administration of L-365,260, a CCK_B receptor antagonist, in the doses of 1 μg, 10 μg or 100 μg (Figure 3B) had no effect on the response of vagal efferent activity to 0.1 nmol CCK-8 (treatment × time interaction $F(3,33)=0.24$, $P=1.0$).

Effect of exogenous i.v. CCK-8 on vagal efferent activity in animals with total subdiaphragmatic vagotomy

To determine the contribution of subdiaphragmatic vagal afferent activity to the responses of the vagal efferent nerve, shown in Figure 2, the contralateral (dorsal) subdiaphragmatic vagal nerve was additionally cut. When injected 0.5 h after total vagotomy, 0.1 and 1 nmol doses of CCK-8 still produced a significant dose-dependent decrease in the gastric vagal efferent nerve activity (Figure 4) (treatment × time interaction $F(3,21)=5.6$, $P<0.001$), although the decrease in nerve activity was smaller than in the conditions where the subdiaphragmatic vagal afferent nerve was left intact. With 0.1 nmol CCK-8 the activity decreased to 82±6% of pre-administration control after 30 s and to 81±5% after 60 s, and with 1 nmol CCK-8 to 56±2% after 30 s and to 59±4% and 60 s. The activity returned to its pre-administration level 2 min after the administration of 0.1 nmol CCK-8 and 3.5 min after 1 nmol CCK-8. The mean latency of the response to CCK-8 was significantly prolonged (1 nmol CCK, 16.7±1.1 s, $P<0.01$) after the cutting of the dorsal subdiaphragmatic vagal nerve. Neither saline nor 0.01 nmol CCK-8 affected vagal efferent nerve activity (Figure 4).

Effect of exogenous i.v. CCK-8 on vagal efferent activity in animals with total subdiaphragmatic vagotomy after i.v. pretreatment with either CCK_A or CCK_B receptor antagonist

To study the contribution of the CCK receptors to the response of vagal efferent activity in rats with total subdiaphragmatic vagotomy, CCK_A or CCK_B receptor antagonist was administered prior to injection of 0.1 nmol CCK-8.

Intravenous injection of L-364,718, a CCK_A receptor antagonist in dose of 1 μg, 5 min prior to the injection of 0.1 nmol CCK-8 partially blocked the response of vagal

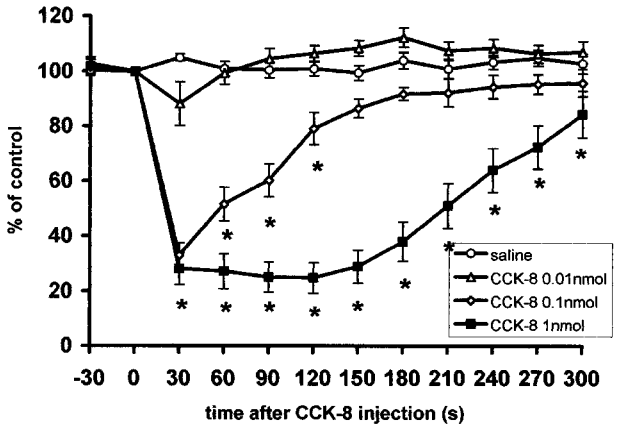

Figure 2 Summarized responses of mass activity in the gastric vagal efferent nerve in rats with partial vagotomy to i.v. administration of CCK-8; per cent of the pre-administration control values, mean± s.e.m., $n=7-8$. Time of the injection 0. *$P<0.05$ between CCK-8 and saline treated groups.

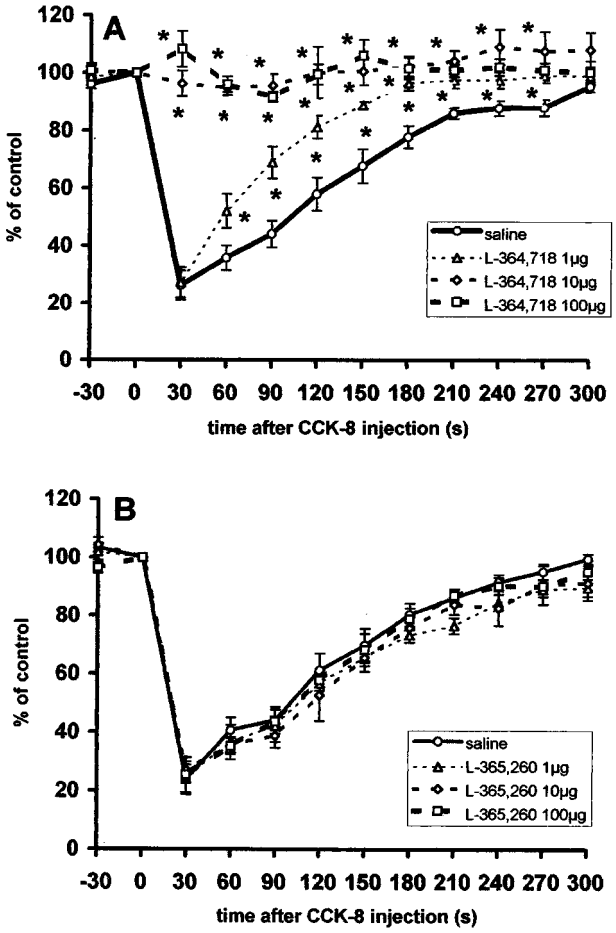

Figure 3 Summarized responses of the gastric vagal efferent nerve activity in rats with partial vagotomy to i.v. administration of 0.1 nmol CCK-8 5 min following i.v. injection of saline or L-364,718, a CCK_A receptor antagonist (A) and saline or L-365,260, a CCK_B receptor antagonist (B). Percentage of the pre-administration control values, mean±s.e.m., $n=6$. Time of the injection 0. *$P<0.05$ between saline and antagonist pre-treated groups.

efferent activity in comparison to that of CCK-8 injected after saline (Figure 5A). Larger doses of the antagonist (10 and 100 μg) completely blocked the response (treatment × time interaction $F(3,21)=3.9$, $P<0.001$).

However, the effects of 0.1 nmol CCK-8 were not attenuated by the prior administration of L-365,260, a CCK_B antagonist, in the doses of 1, 10 or 100 μg (Figure 5B) (treatment × time interaction $F(3,21)=0.75$, $P=0.77$).

Effect of exogenous i.v. CCK-8 on vagal efferent activity in animals with total subdiaphragmatic vagotomy after i.c.m. pretreatment with CCK_A receptor antagonist

The vagal efferent activity response to 0.1 nmol CCK-8 was studied at different time intervals after the i.c.m. injection of CCK_A receptor antagonist (1 μg) in animals with total subdiaphragmatic vagotomy. The reproducibility of the response of the vagal efferent activity to injections of CCK-8 at 10 min intervals was confirmed in control experiments. Ten to twenty minutes after the application of CCK_A receptor antagonist the response of vagal efferent activity to CCK-8 was completely blocked (treatment × time interaction $F(1,7)=3.04$, $P=0.007$), (Figure 6). The response of i.v. CCK-8 returned after 40–50 min after i.c.m. injection of CCK_A receptor antagonist (data not shown).

Effect of exogenous i.v. CCK-8 on the afferent activity of supradiaphragmatic vagal nerves

In order to elucidate the contribution of supradiaphragmatic vagal afferents in the response of vagal gastric efferent activity to CCK-8, the response of vagal afferents at the cervical level was recorded after total subdiaphragmatic vagotomy (Figure 7). No significant effect of 1.0 nmol CCK-8 was found when compared to the saline treated group.

Discussion

The results show that administration of exogenous 0.1–1.0 nmol CCK-8 (ca 0.35–3.5 μg kg^{-1}) significantly decreased the gastric vagal efferent nerve activity. This effect of CCK-8 on gastric vagal efferent activity was mediated by CCK_A receptors, localized both peripherally and centrally.

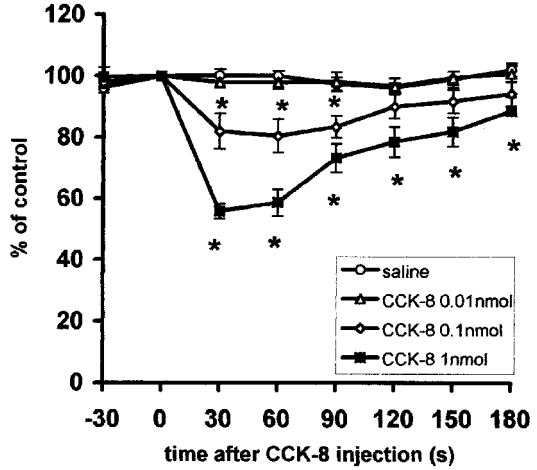

Figure 4 Summarized responses of mass activity in the gastric vagal efferent nerve in rats with total vagotomy to i.v. administration of CCK-8; per cent of the pre-administration control values, mean ± s.e.m., $n=8$. Time of the injection 0. $^*P<0.05$ between CCK-8 and saline treated groups.

In the present study exogenous CCK-8 induced a decrease of gastric vagal efferent activity and this effect of CCK-8 was significantly diminished after total subdiaphragmatic vagotomy, suggesting that the main site of action of CCK-8 was the subdiaphragmatic vagal afferents. These results are supported by Schwartz *et al*. (1994) and Kurosawa *et al*. (1997) who have demonstrated that intravenous injection of CCK-8 increases the vagal gastric afferent activity dose-dependently. The present vago-vagal neural mechanism underlies the reflex responses to CCK-8 of the gastric motility and emptying (Raybould & Taché, 1988; Grundy *et al*., 1995; Rogers *et al*.,

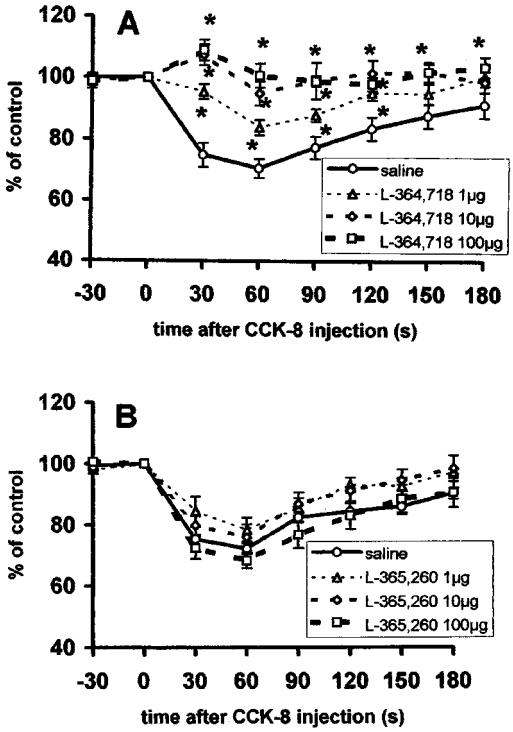

Figure 5 Summarized responses of the gastric vagal efferent nerve activity in rats with total vagotomy to i.v. administration of 0.1 nmol CCK-8 5 min following i.v. injection of saline or L-364,718, a CCK_A receptor antagonist (A), and saline or L-365,260, a CCK_B receptor antagonist (B). Percentage of the pre-administration control values, mean ± s.e.m., $n=6-7$. Time of the injection 0. $^*P<0.05$ between saline and antagonist pre-treated groups.

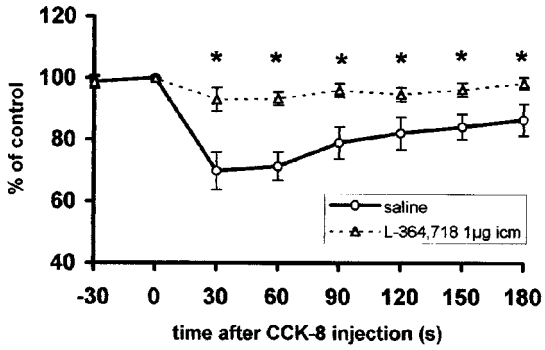

Figure 6 Summarized responses of the gastric vagal efferent nerve activity in rats with total vagotomy to i.v. administration of 0.1 nmol CCK-8 10–20 min following i.c.m. injection of saline or L-364,718, a CCK_A receptor antagonist. Percentage of the pre-administration control values, mean ± s.e.m., $n=6$. Time '0' indicates the time of injection of CCK-8. $^*P<0.05$ between saline and L-364,718 pre-treated groups.

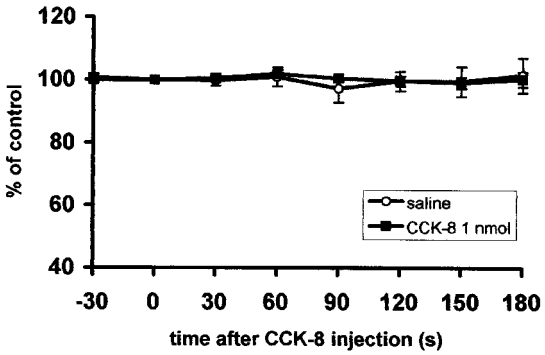

Figure 7 Summarized responses of mass activity in the cervical vagal afferent nerve in rats with total subdiaphragmatic vagotomy to i.v. administration of CCK-8; per cent of the pre-administration control values, mean ± s.e.m., $n=8$. Time of the injection 0.

1995), although the results can not exclude the possibility that the intact contralateral subdiaphragmatic vagal efferent nerve augments the response of the recorded efferent nerve activity to CCK-8 *via* some unknown mechanisms.

The finding that CCK-8 still had an effect on efferent activity after total subdiaphragmatic vagotomy suggests that CCK-8 also might stimulate supradiaphragmatic vagal afferents, and/or act directly on central vagal sites. We had excluded the involvement of supradiaphragmatic vagal afferents in the response of gastric vagal efferents to exogenous CCK-8, since intravenous injection of 1 nmol of CCK-8, a dose significantly affecting both subdiaphragmatic vagal afferent and efferent activity, had no effect on the activity of supradiaphragmatic vagal afferents. Although it is generally accepted that CCK does not readily cross the blood-brain barrier in the time course of the current experiments (Oldendorf, 1981), recent studies suggest that CCK and related peptides may have access to specific brain sites where the blood-brain barrier is incomplete (Harro *et al*., 1993; Hagino & Moroji, 1994). In addition, larger doses of CCK-8 (4–16 $\mu g\ kg^{-1}$) may activate non-vagal sympathetic afferents as reduction in food intake has been observed in rats with subdiaphragmatic vagal deafferentation (Moran *et al*., 1997).

CCK-8 exerts its physiological effects *via* either CCK_A or CCK_B receptors. Studies by Schwartz *et al*. (1994) and Kurosawa *et al*. (1997) demonstrated that the gastric vagal afferents are activated *via* CCK_A receptors, indicating that the profound decrease in the gastric vagal efferent activity in animals with partial subdiaphragmatic vagotomy is mainly produced *via* stimulation of the CCK_A receptors located on the vagal afferent terminals (Zarbin *et al*., 1981).

In order to further determine the type of CCK receptors involved in the inhibition of gastric vagal efferent nerve activity and the localization of the receptors being activated in rats with total subdiaphragmatic vagotomy, CCK_A (i.v. or i.c.m.) or CCK_B (i.v.) receptor antagonist were injected prior to the administration of CCK-8. Systemically administered L-364,718 and L-365,260 have been shown to be able to cross the blood-brain barrier and to reduce the central responses of peripherally administered CCK-8 and CCK-8-related peptides (Hagino & Moroji, 1994; Ladurelle *et al*., 1997; Lin & Lin, 1990). However, this occurs slowly and is unlikely to account for the effects observed in current experiments. Intravenous injection of a CCK_A receptor antagonist blocked the response of vagal efferent activity while that of a CCK_B receptor antagonist had no effect on the response, indicating that the effect of CCK-8 on vagal efferent activity after total vagotomy was mediated through CCK_A, but not CCK_B receptors. Moreover, i.c.m. administration of 1 μg of the CCK_A receptor antagonist, a dose which showed a partial block when administered intravenously, completely blocked the response of the vagal efferent activity to peripherally administered CCK-8. These results suggest that the response of the vagal efferent activity to CCK-8 in rats with total subdiaphragmatic vagotomy are mainly mediated *via* the centrally located CCK_A receptors (Branchereau *et al*., 1993), possibly those in area postrema etc. where the blood-brain barrier is lacking (Gross *et al*., 1990; Mönnikes *et al*., 1997; Moran *et al*., 1986). In accordance with our findings, it has been shown (Mönnikes *et al*., 1997) that the activation of some of the nuclei in the brain such as the paraventricular nucleus, NTS and especially the area postrema by systemic administration of CCK-8 was not totally dependent on the vagal afferent excitation, suggesting the contribution of central CCK receptors. The central CCK_A receptor would further augment the responses of the vagal efferent nerve activity in animals with intact vagal afferents (Barber *et al*., 1995).

Conclusion

The effect of exogenous CCK-8 on vagal efferent activity was realized mainly through peripheral CCK_A receptors located on the subdiaphragmatic vagal afferents, but not through CCK_B. The response of the efferent activity can be partly mediated through the central CCK_A receptors. Our present results are in line with earlier studies showing that CCK_A receptors play a major role in regulating the afferent and efferent gastric vagal activity in response to CCK-8. These neural mechanisms underlie the involvement of CCK-8 in the reflex regulation of gastric motility, emptying, and acid secretion.

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