Interleukin-1 increases activity of the gastric vagal afferent nerve partly via stimulation of type A CCK receptor in anesthetized rats

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Abstract

The response of mass activity of the gastric vagal afferent nerve to intravenous administration of interleukin-1β (IL-1β) and the involvement of cholecystokinin CCK in the response were investigated in pentobarbital-anesthetized rats. Intravenous administration of 2 μg·kg⁻¹ of IL-1β caused an increase in the afferent activity, which reached 150% of control activity by 30 min after administration and persisted for more than 80 min. The increase in the nerve activity was significantly reduced in animals pretreated with a type A CCK receptor antagonist. IL-1β also significantly increased the CCK concentration in systemic blood. Furthermore, it was confirmed that intravenous administration of CCK produced an increase in the nerve activity via the type A CCK receptor. These findings suggest that systemically applied IL-1β increases CCK concentration in systemic blood secreted from mucosal endocrine cells of the small intestine, and that in turn CCK in the gastric blood flow augments or partly participates in the IL-1β-induced excitation of the gastric vagal afferent nerve via stimulation of the type A CCK receptor in the stomach. A possible involvement of IL-1-related excitation of the gastric vagal afferent nerve in IL-1-induced anorexia is discussed.

Keywords: Interleukin-1; CCK; Type A CCK receptor; Gastric vagal afferent nerve; Rat

1. Introduction

During infectious illness, there are general symptoms such as fever, somnolence and anorexia [5,9,17]. All of these symptoms are thought to be mediated by interleukin-1 (IL-1), a 17.5 kDa protein that is rapidly secreted from immunologically activated monocytes/macrophages. Daun and McCarthy [3] have recently shown that the systemic administration of IL-1 induces anorexia in rats and that the anorexia is partly blocked by pretreatment with the systemic administration of a type A cholecystokinin (CCK) receptor antagonist, suggesting that the IL-1-induced anorexia is partly mediated via CCK.

CCK is released into the systemic blood from mucosal endocrine cells of the small intestine in response to components of a meal, such as fatty acid and protein. The released CCK causes well-known actions, i.e. contraction of the gall bladder and pancreatic enzyme secretion. In addition, CCK mediates satiety via the vagal afferent nerves. It has been postulated that CCK in the gastric blood flow excites the gastric vagal afferent nerve by stimulating the type A CCK receptor located at the nerve terminals of the vagal afferents and at the pyloric sphincter [11,12,20], and that the afferent activity conveys satiation signals to the ‘satiety center’ of the brain, resulting in the inhibition of feeding behavior [18,19]. The blockade of the type A CCK receptor by a systemic administration of L-364,718 or Devazepide has been shown to reduce CCK-induced hypophagia [6,10].

Taken together, these findings suggest that systemically applied IL-1 may increase the CCK concentration in systemic blood secreted from mucosal endocrine cells in the small intestine and influence the activity of the gastric vagal afferent nerve via stimulation of the type A CCK receptor in the stomach. To investigate this possibility, we examined the responses of both plasma CCK and the vagal
gastrointestinal afferent activity to intravenous administration of IL-1 with or without pretreatment with a type A CCK receptor antagonist in anesthetized rats. We also studied the responses of the gastric vagal afferent activity to intravenous administration of CCK, by recording the mass afferent activity of vagal nerves.

2. Materials and methods

Experiments were performed on 56 male rats (Sprague–Dawley, 240–360 g) anesthetized with pentobarbital sodium (60–70 mg·kg⁻¹, i.p., Apoteksbolaget, Umeå, Sweden). Animals were food-deprived for 1 day before the experiment.

The trachea was catheterized, and the respiration of the animal was artificially maintained with a respirator (Model 683, Harvard, MA, USA). The femoral vein was cannulated for intravenous administration of substances such as CCK and IL-1β. The jugular vein was also cannulated for constant infusion of pentobarbital and a muscle relaxant, gallamine triethiodide (Sigma Chemical, St. Louis, MO, USA). Blood pressure was monitored continuously from the femoral artery and maintained above 90 mmHg systolic by administration of 4% Ficoll 70 (Pharmacia Fine Chemicals AB, Uppsala, Sweden). Rectal temperature was maintained at 37.5 ± 0.1°C by a heating pad and an infrared lamp (ATB-1100, Nihon-Kohden, Tokyo, Japan).

All surgical procedures mentioned above were usually finished 1 h after the initial injection of anesthetic. A mixed solution of pentobarbital (10–20 mg·kg⁻¹·h⁻¹) and gallamine triethiodide (10–20 mg·kg⁻¹·h⁻¹) was then administered intravenously by an infusion pump (Model STC-527, Terumo, Tokyo, Japan). During the experiment, the depth of anesthesia was routinely judged by observing the fluctuation of the blood pressure of the animal.

Fig. 1. Responses of mass activity of the gastric vagal afferent nerve to intravenous (i.v.) administration of IL-1β (2 µg·kg⁻¹). (A,B) Sample recordings from two rats. The nerve activity was counted every 20 s. (C) Summarized responses in 12 rats. Ordinates: magnitude of the responses; 1-min count of the nerve activity is expressed as percentage of the pre-administration control values; Abscissa: time in min. 0 indicates the time of the injection. Circles: IL-1β injected (n = 6). Triangles: vehicle injected (n = 6). Each point and vertical bar show mean ± S.E.M. * P < 0.05, ** P < 0.01, determined between the IL-1β-treated group and the vehicle-treated group.

2.1. Drug treatment

Sulfated cholecystokinin octapeptide (CCK-8) (Peninsula Lab. Europe, Merseyside, UK) was dissolved in saline containing 0.1% bovine serum albumin (BSA) and cumulatively administered at doses of 0.01, 0.1 or 1 nmol in 0.2 ml. L-364,718 (Merck Research Lab., NJ, USA), a type A CCK receptor antagonist, and L-365,260 (Merck), a type B CCK receptor antagonist, were first dissolved in 50% dimethyl sulfoxide at a concentration of 12.5 µmol·ml⁻¹, and then diluted with saline. These antagonists were cumulatively injected at doses of 0.25 (L-364,718 alone), 1.25, 25 or 250 nmol in 0.2 ml solution 5 min before the administration of CCK-8 (0.1 nmol).

Recombinant human IL-1β (Bachem Feinchemikalien AG, Bubendorf, Switzerland) was diluted in 0.2 M Tris-HCl buffer, pH 7.4 at 25°C, containing 0.2% BSA, and injected at a dose of 2 µg·kg⁻¹. In six rats, 250 nmol of L364,718 was injected 5 min before the administration of IL-1β (2 µg·kg⁻¹). In three rats, heat-inactivated IL-1β (2 µg·kg⁻¹; 70°C for 30 min) was also administered. All these substances were intravenously injected over a 20-s period.

2.2. Recording of mass activity of the gastric vagal afferent nerve

The abdomen was opened by a midline incision. A few anterior subdiaphragmatic vagal nerve branches innervating the stomach were dissected under a binocular microscope and cut about 1 cm proximal to the entrance of the stomach. Both the anterior and posterior subdiaphragmatic vagal trunks were cut to avoid the involvement of vago-vagal reflexes. In three rats, gastric sympathetic nerves were also crushed. The peripheral cut segment of the nerve branch was placed in contact with a pair of bipolar platinum wire electrodes and the mass afferent activity was
amplified (Nihon Kohden S-0476, Tokyo, Japan, time constant: 0.01 s). The number of the afferent activity was counted by a pulse counter after passing through a window discriminator (ME-1100, Nihon Kohden) and recorded on a polygraph. After 30 min of stable activity had been recorded, substances such as CCK and IL-1β were intravenously administered. For the responses to IL-1β, a 1-min count of the afferent activity was measured every 10 min until 80 min after the onset of the administration, and expressed as the percentage of the pre-administration control value 1 min after the onset of the administration. For the responses to CCK-8, the 20-s count of the afferent activity was measured between 30 and 50 s after the onset of the administration, and expressed as the percentage of the pre-administration control value (20-s count of the nerve activity immediately before the onset of the CCK-8 administration).

### 2.3 Measurement of plasma CCK

Heparinized (60 IU/rat, i.v.) blood samples were taken by cardiac puncture with a 10-ml syringe (needle size: 22 gauge), 30 min after the intravenous administration of IL-1β (2 μg·kg⁻¹) or its vehicle. The blood was collected in chilled plastic tubes containing 10 IU·ml⁻¹ aprotinin (Bayer AB, Stockholm, Sweden). The samples were centrifuged immediately and the plasma was frozen (−20°C).

Before the determination of CCK, the peptide was separated from plasma proteins using SEP-PAK C-18 cartridges (Waters Assoc., Milford, MA, USA) as described previously [8]. For the determination of CCK, the purified samples were dissolved in half of their original volume and thereby concentrated twice. A correction factor was introduced later.

Radioimmunoassay (RIA) of CCK was performed using antibody A99 (Euro-Diagnostica AB, Malmö, Sweden), which specifically recognizes the sulfated form of cholecystokinin 26–33. A standard curve was prepared using sulfated CCK-8 Peninsula diluted in assay buffer. Of the antibody, 50 μl (final dilution 1:250) was incubated at room temperature together with 100 μl of standard or purified sample dissolved in RIA buffer and 125I-labeled CCK-8 (Du Pont NEN, Boston, MA, USA) for 24 h. Separation of the bound from the unbound fraction was done by incubating the samples together with a second antibody immunoadsorbent (Kabi Pharmaceuticals AB, Uppsala, Sweden) for 30 min. The limit for detection of CCK was 0.25 fmol per tube. Intra- and interassay coefficients of variation were 8 and 11%, respectively. Finally, the plasma concentration of CCK was normalized into the value per 300 g body weight.

### 2.4 Statistical analysis

Data were expressed as mean ± S.E.M. Comparisons of group differences were made using analysis of variance followed by Dunnett’s t-test. Data on plasma CCK-LI were analyzed by unpaired t-test between animals that
received an IL-1β injection and those that did not. Probability values less than 5% were considered significant.

3. Results

3.1. Responses of the gastric vagal afferent activity to IL-1β administration

As shown in Fig. 1A and C, intravenous administration of 2 μg·kg⁻¹ of IL-1β resulted in marked but delayed increases in the mass activity of the gastric vagal afferent nerve. The response generally began within several minutes of administration, and reached a maximum value (148 ± 8% of control) after 30 min (n = 6 rats). It then began a slow decline, but was still significantly increased (140 ± 11% of control) after 80 min. Intravenous injection of heat-inactivated IL-1β (2 μg·kg⁻¹ in 3 rats) or vehicle (in 6 rats, Fig. 1B and C) did not cause any significant change in the nerve activity.

The activation of the gastric vagal afferent nerve by intravenous administration of 2 μg·kg⁻¹ of IL-1β was also observed in the 3 rats whose gastric sympathetic nerves were crushed.

3.2. Effect of a type A CCK receptor antagonist on IL-1β-induced increases in the gastric vagal afferent activity

As shown in Fig. 2A and C, intravenous administration of 2 μg·kg⁻¹ of IL-1β caused a long-lasting increase in the mass activity of the gastric vagal afferent nerve in rats pretreated with intravenous administration of 250 nmol of L-364,718, the type A CCK receptor antagonist. However, the response was significantly smaller than the one in animals without the pretreatment (Fig. 2C). Intravenous administration of L364,718 alone had no effect on the afferent activity (Fig. 2B and C).

3.3. Response of plasma concentration of CCK to IL-1β

The plasma concentration of CCK-like immunoreactivity (CCK-LI) was measured. Systemic blood was taken 30 min after the intravenous administration of 2 μg·kg⁻¹ of IL-1β when the response of the gastric vagal afferent nerve...
activity to the same dose of IL-1β showed the maximum increase (see Fig. 1). As shown in Fig. 3, administration of IL-1β significantly increased the plasma concentration of CCK-LI (n = 5; 2.87 ± 0.57 pM), compared with that of vehicle treatment (n = 6; 1.58 ± 0.22 pM) (P < 0.05).

3.4. Responses of the gastric vagal afferent activity to CCK-8 administration

As shown in sample recordings in Fig. 4A–C, intravenous administration of 0.01, 0.1 and 1 nmol of CCK-8 increased the mass activity of the gastric vagal afferent nerve in a dose-dependent manner. The increase in the activity reached a maximum about 30–50 s after the onset of the administration. As shown in Fig. 4D, the average maximum increases reached 172 ± 16% and 275 ± 29% of control activity at dosages of 0.1 nmol (P < 0.05) and 1 nmol (P < 0.01) of CCK-8, respectively (n = 7 rats). Administration of 0.01 nmol of CCK-8 did not produce any significant changes in the nerve activity.

This increase in gastric vagal afferent activity in response to 0.1 nmol of CCK-8 was antagonized dose-dependently by the pretreatment with intravenous administration of 25–250 nmol of L-364,718, the type A CCK receptor antagonist (Fig. 5), but not by the intravenous pretreatment with 2.5–250 nmol of L-365,260, the type B CCK receptor antagonist (Fig. 6).

Additional experiments confirmed that intravenous administration of 250 nmol of L-364,718 completely blocked the increased response of the gastric vagal afferent activity to intravenous injection of 0.1 nmol of CCK-8 during the 80-min observing period.

4. Discussion

The present results demonstrated that the intravenous injection of IL-1β increased the plasma concentration of CCK-LI and activated the gastric vagal afferent nerve partly via stimulation of the type A CCK receptor. We also confirmed the excitation of the gastric vagal afferent nerve in response to intravenous administration of CCK via the type A CCK receptor, not via the type B CCK receptor. These findings suggest that IL-1β increases the secretion of CCK from mucosal endocrine cells of the small intestine into the systemic blood, and that in turn CCK in the gastric blood flow partly participates in the IL-1β-induced activation of the gastric vagal afferent nerve via stimulation of the type A CCK receptor in the stomach. It is also possible that CCK sensitizes the response of the gastric vagal afferent activity to IL-1, since CCK is known to sensitize the vagal afferent response to gastric distention [15].

Responses of the gastric vagal afferent activity to CCK in rats [4,15,16] and in ferrets [1,2] have been reported by several investigators. Schwartz et al. [16] have recently shown that the mechano-sensitive gastric afferents in rats recorded from the single cervical vagal nerve are excited in response to CCK-8, and that the increases are antagonized by a type A CCK receptor antagonist, but not by a type B CCK receptor antagonist. We evaluated the mass activity (including both mechano- and chemo-sensitive) of the abdominal gastric vagal afferent nerve in rats. Our results were basically consistent with the study by Schwartz et al. [16]. However, the mean response of the mechano-sensitive afferents to 0.1 nmol of CCK-8 in the experiment by Schwartz et al. (ca. 800% of pre-administration control activity) was bigger than the mean response of mass afferents observed in our present experiment (ca. 170% of pre-administration control activity). This suggests that the spontaneous activities of the gastric mechano-sensitive afferents selectively increase in response to administered CCK, and that the responsiveness of the gastric chemosensitive afferents to administered CCK is minimal in rats, although the chemosensitive afferents are sensitive to CCK in ferrets [1].

The efferent activity of the sympathetic nerve changes in response to the administration of IL-1 [13]. In the present experiment, the mass activity of the gastric vagal afferent nerve was recorded in the animals whose bilateral abdominal vagal nerves were surgically cut, and the increases in the nerve activity were confirmed in the animals whose gastric sympathetic nerves were additionally crushed. These results demonstrate that the increased re-

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Fig. 6. Effects of L-365,260, a type B CCK receptor antagonist, on the responses of mass activity of the gastric vagal afferent nerve to intravenous (i.v.) administration of cholecystokinin octapeptide (CCK; 0.1 nmol). (A,B) Sample recordings from one rat. (C) Summarized responses in 4 rats. See Fig. 5 for other details.
response of the gastric vagal afferent activity to IL-1 is not secondary to changes in the efferent activity of the vagus and/or sympathetic nerves via stimulation of the central nervous system by IL-1.

The excitation of the subdiaphragmatic vagal afferent nerve inhibits the nociceptive tail flick reflex in rats [21]. Thus, in addition to induction of the satiating signal, IL-1- or CCK-induced activation of the gastric vagal afferent nerve may have another function related to analgesia.

IL-1 induces anorexia, and the anorexia is partly blocked by systemic administration of a type A CCK receptor antagonist [3], suggesting a contribution of CCK to IL-1-induced anorexia. CCK decreases food intake and gastric emptying through an excitation of the vagal afferent nerve via the type A CCK receptor [6,10,18,19]. In the present study, we have shown that IL-1 excites the gastric vagal afferent nerve partly via stimulation of the type A CCK receptor. Taken together, these findings suggest that the IL-1-induced anorexia is mediated by an excitation of the gastric vagal afferent nerve partly via stimulation of the type A CCK receptor. In contrast, IL-1-induced anorexia is considered by some investigators to be a solely central nervous system-mediated response, from studies using intracerebroventricular injection of IL-1 [14,22]. However, the existence of a blood–brain barrier to macromolecules such as IL-1 raises a question as to how circulating IL-1 gains initial access to the brain parenchyma to exert its effects. Recent studies have suggested that the activation of the vagal afferent nerve can play a key role in changing the neuronal activity of the central nervous system. For example, subdiaphragmatic vagotomy completely blocked the induction of c-fos protein following intraperitoneal injections of lipopolysaccharide [23]. Many IL-1-responsive cells were found in the nucleus solitary tract where the primary vagal afferent nerves terminate [7]. Thus, it is possible that circulating IL-1 primarily activates the vagal afferent nerve and in turn activates various regions of the brain, thereby exhibiting both peripheral (vagually-mediated) and central mechanisms for its anorexic effect in infectious illness.

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