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Role of neuropeptide receptor systems in vanilloid VR1 receptor-mediated gastric acid secretion in rat brain

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Abstract

Previously, we reported that the injection of capsaicin into the lateral cerebroventricle (i.c.v.) stimulated gastric acid secretion via vanilloid VR1 receptors and the vagal cholinergic pathways in anesthetized rats. In the present study, we investigated the involvement of receptor systems for neurokinin A, calcitonin gene-related peptide (CGRP) and glutamate in the vanilloid VR1 receptor-mediated response. The i.c.v. injection of neurokinin A (30 nmol) stimulated gastric acid secretion in the presence of *cis*-2-(diphenylmethyl)-*N*-[(2-iodophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine oxalate (L-703606, a tachykinin NK_1 receptor antagonist, 30 nmol) and the effect was inhibited by cyclo[Gln-Trp-Phe-Gly-Leu-Met] (L-659877, a tachykinin NK_2 receptor antagonist, 30 nmol); the values were 145.9±32.3 and 21.1±16.6 μEq HCl per 120 min, respectively. The value in the control group was 14.3±3.8 μEq HCl. The tachykinin NK_2 receptor-mediated secretion was inhibited by i.c.v. injections of antagonists of the CGRP1 receptor (human CGRP fragment 8–37, 15 nmol) and non-*N*-methyl-D-aspartate (non-NMDA)-type glutamate receptor (6-cyano-7-nitroquinoxaline-2,3-dione, 10.9 nmol); the values were 30.8±29.8 and 5.7±16.9 μEq HCl, respectively. Gastric acid secretion induced by the i.c.v. injection of 30 nmol capsaicin (178.4±34.0 μEq HCl) was inhibited by antagonists of tachykinin NK_2 (23.7±6.2) and CGRP1 (21.2±8.5), but not tachykinin NK_1 (181.4±37.0), receptors. The gastric acid secretion induced by capsaicin was decreased by the i.c.v. pre-injection of low doses of neurokinin A or CGRP, which alone had no effect on the secretion. These findings suggest the involvement of tachykinin NK_2, CGRP and non-NMDA receptor systems in the vanilloid VR1 receptor-mediated regulation of gastric acid secretion in the rat brain regions close to the lateral cerebroventricle.

Keywords: Gastric acid secretion; Vanilloid VR1 receptor; Tachykinin; CGRP (Calcitonin gene-related peptide); Glutamate; Central injection; (Rat)

1. Introduction

Many studies concerning the central effects of various neurotransmitters and neuropeptides on gastric acid secretion have revealed mechanisms by which the brain regulates this secretion (Geoghegan and Pappas, 1997; Yang et al., 2000; Hornby, 2001). Previously, we reported that the injection of kainate, *N*-methyl-D-aspartate (NMDA, Tsuchiya et al., 2001) and the agonists of κ-opioid receptors (Ishihara et al., 2001) into the lateral cerebroventricle (i.c.v.) stimulated gastric acid secretion via vagal cholinergic neurons in rats. Glutamate receptors, specifically the non-NMDA type, are involved in the gastric acid secretion induced by κ-opioid receptor activation in the brain region close to the lateral cerebroventricle in rats (Minowa et al., 2003). In addition, we reported that the i.c.v. injection of capsaicin (30 nmol) stimulated gastric acid secretion via the vagal efferent cholinergic pathways in rats (Minowa et al., 2001). Since the secretion induced by capsaicin was inhibited by the i.c.v. injection of capsazepine and ruthenium red, the capsaicin response appeared to be mediated via vanilloid VR1 receptors in the central nervous system (CNS) in rats (Minowa et al., 2001).

Stimulation of primary afferent neurons in the spinal cord and peripheral tissues with capsaicin releases various neurotransmitters (Szallasi and Blumberg, 1999; Willis, 2001). One of these is the tachykinins: substance P, neurokinin A and neurokinin B. In general, substance P displays high affinity for tachykinin NK_1 receptors, and neurokinin A and neurokinin B bind preferentially to tachykinin NK_2 receptors and tachykinin NK_3 receptors, respectively (Severini et al., 2002), although the tachykinins are capable of binding on all tachykinin NK_{1-3} receptors. Calcitonin gene-related pepti-

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des (CGRPs) are also released from capsaicin-sensitive neurons in various peripheral tissues (Poyner et al., 2002). The i.c.v. and/or central injections of tachykinins or CGRPs produced various pharmacological responses (Poyner et al., 2002; Severini et al., 2002; Holden et al., 2002; Dhillo et al., 2003). Tachykinins are contained in the hypothalamic neurons (De Laurentiis et al., 2003) and the existence of their receptors has been confirmed in the various brain regions including the hypothalamus (Severini et al., 2002). The calcitonin family of peptides including CGRP and the components of CGRP receptors are expressed in the CNS including various hypothalamic nuclei (Oliver et al., 2001; Poyner et al., 2002, Ma et al., 2003). The decrease of respiratory frequency induced by the injection of capsaicin into the nucleus of the solitary tract (Mazzone and Geraghty, 1999) and the antidiuresis induced by the injection of capsaicin into the hypothalamus (Tsushima and Mori, 1999) were inhibited by the central injection of the antagonists for tachykinin NK receptors. Stimulation of vanilloid VR1 receptors caused the release of CGRPs from slices of rat dorsal spinal cord (Tognetto et al., 2001). However, the role of tachykinins and CGRPs in vanilloid VR1 receptor-mediated regulation of gastric acid secretion in the CNS has not been established. Although central injections of these neuropeptides showed inhibitory effects on gastric acid secretion (Taché et al., 1984; Lenz et al., 1984, 1985a,b, 1989; Okuma and Osumi, 1991; Guidobono et al., 1994; Improta et al., 1997; Yang and Taché, 1997), the role of the receptor subtypes in the hypothalamic regions has not been well established.

In the present study, we investigated the role of the neuropeptides in gastric acid secretion and their involvement in vanilloid VR1 receptor-mediated acid secretion in the hypothalamic regions in rats, using gastric perfusion. Our findings suggest that tachykinin NK_2 receptors and CGRP1 receptors systems in the CNS regulate gastric acid secretion positively and that there is cross-talk between vanilloid VR1 receptors, neurokinin NK_2 receptors and CGRP1 receptors in the hypothalamic regions of rats.

2. Experimental procedures

2.1. Animals

Male Wistar rats (Takasugi Exp. Animals, Kusakabe, Japan) weighing 220–400 g were used. The animals were housed under controlled environmental conditions (temperature 24±2 °C and light between 7:00 a.m. and 7:00 p.m.) and fed commercial rat chow (Oriental Yeast, Japan). The rats were fasted overnight before each experiment with free access to water. Animal experiments were performed in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society.

2.2. Drugs

Neurokinin A, neurokinin B and CGRP (rat and human) were purchased from Peptide Institute (Osaka, Japan). Capsaicin was from Wako (Osaka, Japan). *Cis*-2-(diphenylmethyl)-*N*-[(2-iodophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine oxalate (L-703606), cyclo[Gln-Trp-Phe-Gly-Leu-Met] (L-659877), human CGRP fragment 8–37 (hCGRP-(8–37)), [β-Ala^8]neurokinin A fragment 4–10 ([β-Ala^8]neurokinin A-(4–10)), succinyl-[Asp^6, *N*-Me-Phe^8]substance P fragment 6–11 (senktide) and 6-cyano-7-nitroquinoxaline-2,3-dione sodium salt (CNQX) were obtained from Sigma (St. Louis, MO, USA). NMDA and kainate were purchased from RBI (Natic, MA, USA). Capsaicin, L-703606, L-659877, [β-Ala^8]neurokinin A-(4–10) and senktide were dissolved in a minimum of Tween 80 (Nakarai Tesque, Kyoto, Japan) and diluted with saline. The final concentration of Tween 80 was 1% (v/v). Other agents were dissolved with saline. The doses of the antagonists and/or agonists for i.c.v. injection were selected based on the following studies (Jolicoeur et al., 1992; Taché, 1992; Van Rossum et al., 1997; Tsuchiya et al., 2001; Holden et al., 2002; Dhillo et al., 2003; Minowa et al., 2003). The final pH values of these solutions were pH 4.5–6.5. The i.c.v. injection of saline or the vehicle showed no effect on gastric acid secretion.

2.3. Cannulation for central injection to the lateral cerebroventricle

Cannulation for central injection was performed as previously reported (Minowa et al., 2001, 2003). Briefly, the rats were anesthetized with urethane (1.35 g per kg, i.p.). Then, the rats were placed on a stereotaxic instrument, and a 24-gauge guide cannula for the injection of drugs was implanted into the lateral cerebroventricle with the following coordinates: 1.0 mm posterior to the bregma, 1.3 mm right lateral to the midsagittal suture, and 3.8 mm vertical to the surface of the skull with the incisor bar set 3.3 mm below the interaural line. The cannula was secured with dental cement. At the end of the experiments, Evans blue solution was injected to confirm the solution had diffused into the brain regions close to the lateral cerebroventricle.

2.4. Measurement of gastric acid secretion

Each experiment was started at least 1 h after implantation of the cannula. Gastric acid secretion was determined with gastric perfusion methods as previously reported (Minowa et al., 2001, 2003). The trachea was exposed, then cannulated and the esophagus was ligated at the cervical level. After laparotomy, the pylorus was ligated and a dual cannula was inserted into the gastric lumen from the forestomach. The stomach lumen was continuously perfused with saline (adjusted to pH 5.0 with 0.1 N HCl, at 37 °C) at the rate of 1 ml per min through the inlet tube of the dual cannula connected

to the perfusion pump. The stomach was maintained at a pressure of 5 cm H_2O. After the determination of basal acid secretion for 30 min, each test compound was administered in a volume of 5–10 μl over 30 s, using a microliter syringe through a guide cannula positioned in the brain. The antagonists were administered 10 and/or 20 min before the injection of agonists such as neurokinin A and capsaicin. The perfusate flowing from the outlet tube was collected as 10-min fractions with a fraction collector and titrated to pH 5.0 with 0.02 N NaOH. The acid output was expressed in terms of μEq HCl per 10 min and the total acid output for 120 min was shown.

2.5. Statistical analysis

The values are expressed as means±S.E.M. for three to five rats. In the case of multiple comparisons, the statistical significance of differences was determined using a one-way analysis of variance followed by a post hoc test. $P<0.05$ was considered to be statistically significant.

Table 1
Effects of the i.c.v. injection of neurokinin A, [β-Ala^8]neurokinin A-(4–10) and CGRP on gastric acid secretion stimulated by the second injection of capsaicin

Treatment	Vehicle	Neurokinin A	[β-Ala^8] Neurokinin A-(4–10)	CGRP
Acid output after the first injection (μEq HCl per 120 min)				
	−15.9±8.4 (*n*=4)	40.8±29.1 (*n*=4)	−6.5±12.8 (*n*=5)	4.8±23.5 (*n*=4)
Acid output after the second injection of capsaicin				
Capsaicin	178.4±34.1[a] (*n*=6)	59.0±19.1[b] (*n*=4)	0.5±8.9[b] (*n*=3)	38.1±22.1[b] (*n*=4)

Vehicle, neurokinin A (10 nmol per rat, 5 μl), [β-Ala^8]neurokinin A-(4–10) (10 nmol, 5 μl) or CGRP (30 nmol, 10 μl) was injected into the lateral cerebroventricle, and then gastric acid secretion was measured for 120 min. At 120 min after the first injection of the indicated agents, capsaicin (30 nmol, i.c.v.) was injected in the indicated groups. The acid outputs stimulated by the second injection of capsaicin were measured for 120 min. Values represent means±S.E.M. for the indicated number of rats.

[a] $P<0.01$, significantly different from the control group.

[b] $P<0.05$, significantly different from that in the vehicle-treated group.

3. Results

3.1. Stimulatory effect of i.c.v. injection of neurokinin A via tachykinin NK_2 receptor activation on gastric acid secretion

First, we investigated the effect of neurokinin A on gastric acid secretion from urethane-anesthetized rats, using gastric perfusion methods. The injection of neurokinin A into the lateral cerebroventricle (30 nmol per rat, i.c.v.) increased gastric acid secretion in some animals (228.7±30.3 μEq HCl per 120 min, *n*=3), but not in others (11.0±4.3, *n*=3). In addition, the i.c.v. injection of 10 nmol of neurokinin A showed limited and/or marginal effects on the secretion (Table 1). As mentioned in the Introduction, neurokinin A acts on both tachykinin NK_1 and NK_2 receptors. Next, we investigated the effects of selective antagonists, L-703606 (for tachykinin NK_1 receptors) and L-659877 (for tachykinin NK_2 receptors), on the gastric acid secretion induced by neurokinin A. Although the i.c.v. injection of L-703606 (30 nmol) alone showed no effect on gastric acid secretion (Fig. 1A), the injection of neurokinin A (30 nmol) in the presence of L-703606 increased the secretion markedly in all animals tested (Fig. 1B). The secretion began to increase about 30 min after the injection of neurokinin A. The total acid output for 120 min was significantly stimulated by the combination of neurokinin A plus L-703606 (Fig. 1C). The effect of neurokinin A plus 10 nmol of L-703606 on the secretion was marginal. The gastric acid secretion induced by neurokinin A plus L-703606 (30 nmol, respectively) was significantly inhibited in the L-659877 (30 nmol)-treated group (Fig. 1B and C), although 10 nmol of L-659877 did not show an inhibitory effect (data not shown). Vehicle and L-659877 alone showed no effect. Gastric acid secretion induced by the i.c.v. injection of kainate (0.5 nmol) was not inhibited by L-659877 (Fig. 3) and L-703606 (data not shown). In addition, the secretion induced by NMDA (68 nmol, i.c.v.) was not modified by 30 nmol of L-659877; the total acid outputs induced by NMDA in the absence and presence of L-659877 were 64.8±29.6 and 78.3±26.7 μEq HCl per 120 min, respectively (*n*=3).

The i.c.v. injection of [β-Ala^8]neurokinin A-(4–10) (50 nmol, 10 μl), a selective agonist of tachykinin NK_2 receptors, increased gastric acid secretion slightly but significantly (38.8±15.2 μEq HCl per 120 min, *n*=3, $P<0.05$), compared with the control group (−8.4±2.3, *n*=3). The effect of [β-Ala^8]neurokinin A-(4–10) was inhibited by 30 nmol of L-659877 in the two rats tested; the total outputs were 2.1 and −2.2 μEq HCl per 120 min. The i.c.v. injection of 2 (data not shown) and 10 nmol of [β-Ala^8]neurokinin A-(4–10) (Table 1) showed no effect. The i.c.v. injection of neurokinin B (10 nmol, an endogenous agonist of tachykinin NK_3 receptors) and senktide (30 nmol, a selective and synthetic agonist of tachykinin NK_3 receptors) showed no effect on gastric acid secretion; the total acid outputs were 43.3±28.8 and −16.8±33.1 μEq HCl per 120 min (*n*=3), respectively.

3.2. Involvement of CGRP and non-NMDA receptor systems in gastric acid secretion induced by tachykinin NK_2 receptor activation

The i.c.v. injection of 30 nmol of human CGRP increased gastric acid secretion in some animals (68.0±18.6 μEq HCl per 120 min, *n*=3), but not in others (−16.8±5.0, *n*=5). Thus, the effect of 30 nmol of CGRP was marginal (Table 1). The i.c.v. injections of 10 nmol of human CGRP and rat CGRP showed no effect. The i.c.v. injection of 15 nmol of hCGRP-(8–37), an antagonist of CGRP1 receptors, significantly inhibited the gastric acid secretion induced by neuro-

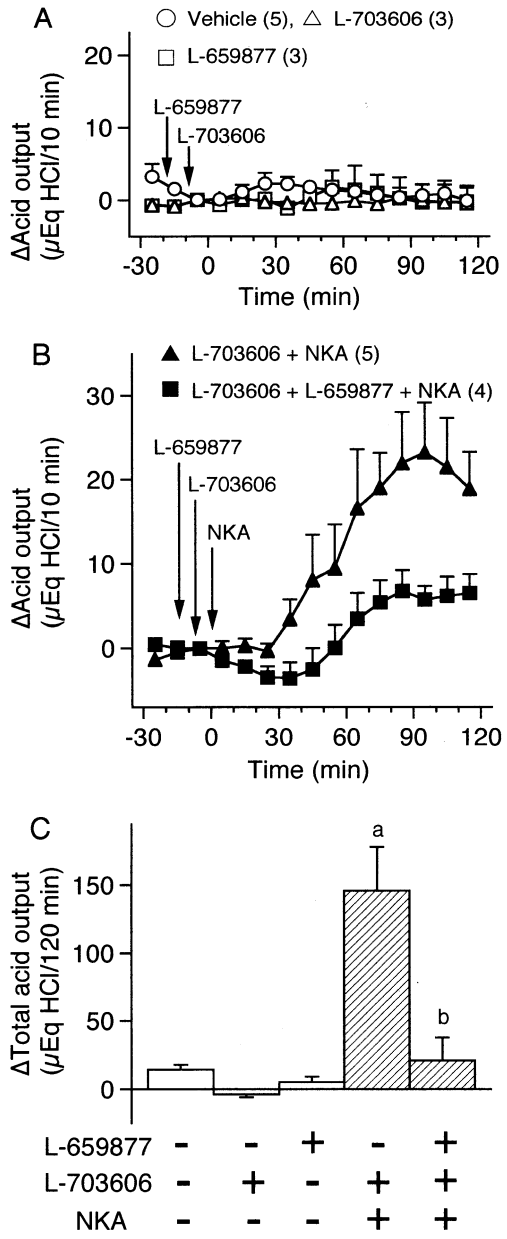

Fig. 1. Stimulatory effect of the i.c.v. injection of neurokinin A plus L-703606 on gastric acid secretion and its inhibition by L-659877. Vehicle (saline, 10 μl,○), L-703606 (30 nmol per rat, 10 μl, △ ▲ ■) or L-659877 (30 nmol, 10 μl, □ ■) was injected into the lateral cerebroventricle at the indicated time before the injection of vehicle (○ △ □) or neurokinin A (NKA, 30 nmol, 10 μl, ▲ ■). Panel (A) shows the effects of the indicated antagonists alone on gastric acid secretion. Panel (B) shows the effects of neurokinin A plus L-703606 in the presence (■) and absence (▲) of L-659877. Each value represents the gastric acid output for 10 min. Panel (C) shows the total acid output for 120 min. Each value and column represents the mean±S.E.M. for three to five rats. [a]$P<0.05$, significantly different from the control (vehicle) group. [b]$P<0.05$, significantly different from the neurokinin A plus L-703606-treated group without L-659877.

kinin A (30 nmol) plus L-703606 (30 nmol) by about half (Fig. 2), although 5 nmol of hCGRP-(8–37) did not show an inhibitory effect. The injection of CNQX (10.9 nmol, an antagonist of non-NMDA receptors) almost completely inhibited gastric acid secretion induced by the i.c.v. injection of neurokinin A (30 nmol) plus L-703606 (30 nmol). The vehicle, hCGRP-(8–37) and CNQX alone showed no effect

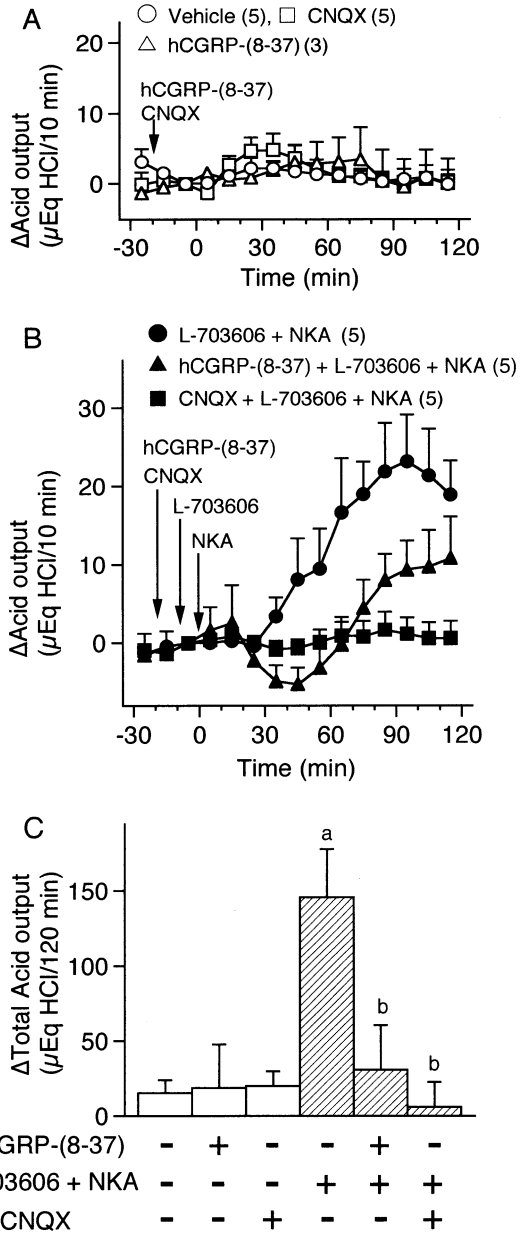

Fig. 2. Inhibitory effect of the i.c.v. injection of hCGRP-(8–37) and CNQX on gastric acid secretion stimulated by neurokinin A plus L-703606. Vehicle (○ ●), hCGRP-(8–37) (15 nmol per rat, 10 μl, △ ▲) or CNQX (10.9 nmol, 5 μl, □ ■) was injected into the lateral cerebroventricle 20 min before the injection of neurokinin A (NKA, 30 nmol) plus L-703606 (30 nmol). L-703606 was injected 10 min before the injection of neurokinin A. Panel (A) shows the effects of the indicated antagonists alone on gastric acid secretion. Panel (B) shows the effects of neurokinin A plus L-703606 in the presence of vehicle (●), hCGRP-(8–37) (▲) or CNQX (■). Each value represents the gastric acid output for 10 min. Panel (C) shows the total acid output for 120 min. Each value and column represents the mean±S.E.M. for three to five rats. [a]$P<0.05$, significantly different from the control group. [b]$P<0.05$, significantly different from the neurokinin A plus L-703606-treated group.

on gastric acid secretion (Fig. 2A). Gastric acid secretion induced by the i.c.v. injection of kainate (0.5 nmol) was not inhibited by hCGRP-(8–37) (Fig. 3).

3.3. Involvement of tachykinin NK_2 and CGRP1, but not tachykinin NK_1, receptor systems in gastric acid secretion induced by vanilloid VR1 receptor activation

As reported previously (Minowa et al., 2001), the i.c.v. injection of capsaicin (30 nmol) stimulated gastric acid secretion in rats (Fig. 4). We investigated the effects of antagonists of tachykinin NK_1 and NK_2 receptors and CGRP1 receptors on gastric acid secretion induced by capsaicin. The capsaicin response was almost completely inhibited in the L-659877- and hCGRP-(8–37)-treated groups, but not in the L-703606-treated group. To further investigate the interactions between vanilloid VR1, tachykinin NK_2 and CGRP1 receptors, we investigated the effect of capsaicin on gastric acid secretion from the neuropeptide-

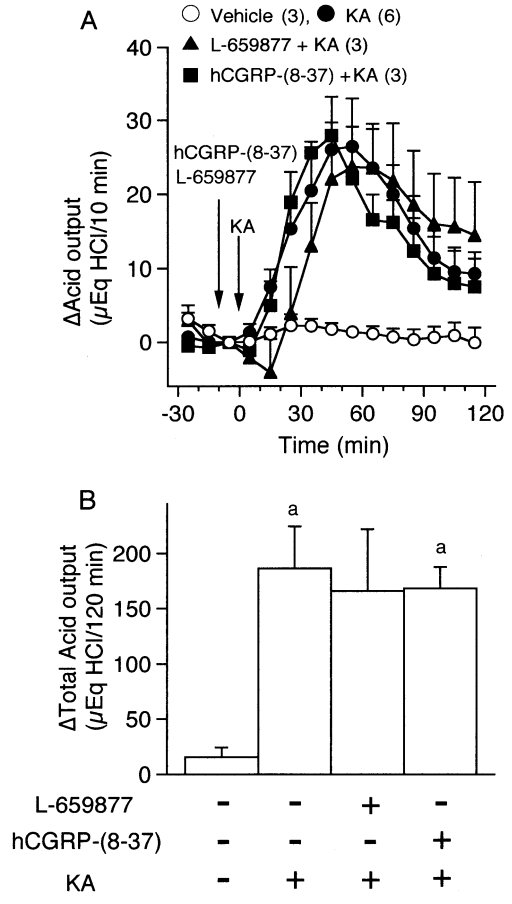

Fig. 3. Effects of the i.c.v. injection of L-659877 and hCGRP-(8–37) on gastric acid secretion stimulated by kainate. Vehicle (○ ●), L-659877 (30 nmol, ▲) or hCGRP-(8–37) (15 nmol, ■) was injected into the lateral cerebroventricle 10 min before the injection of kainate (KA, 0.5 nmol, ● ▲ ■). Panel (A) shows the gastric acid output for 10 min. Panel (B) shows the total acid output for 120 min. Each value and column represents the mean±S.E.M. for three to six rats. [a]$P<0.01$, significantly different from the control group.

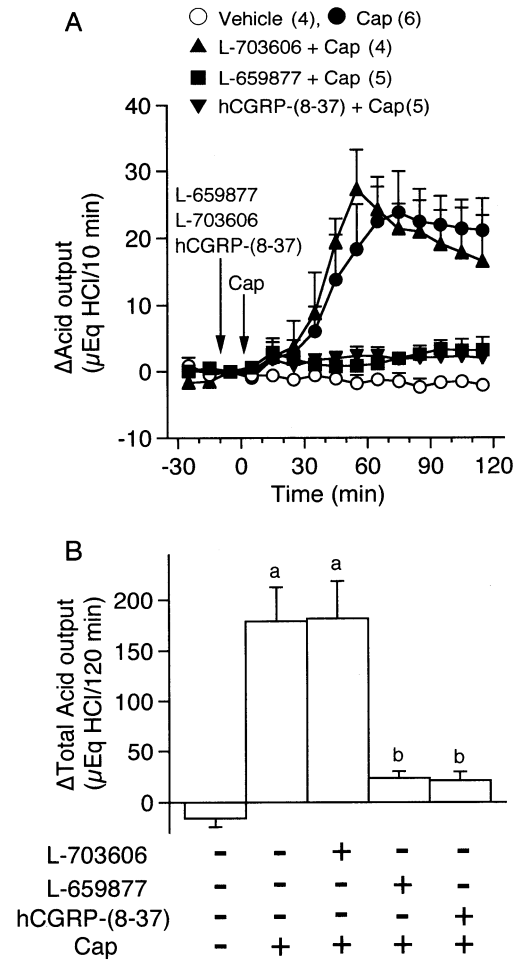

Fig. 4. Inhibitory effects of the i.c.v. injection of antagonists of tachykinin NK_2 and CGRP receptors, but not tachykinin NK_1 receptors, on gastric acid secretion stimulated by capsaicin. Vehicle (○ ●), L-703606 (30 nmol, ▲), L-659877 (30 nmol, ■) or hCGRP-(8–37) (15 nmol, ▼) was injected into the lateral cerebroventricle 10 min before the injection of capsaicin (Cap, 30 nmol per rat, 10 μl, ● ▲ ■ ▼). Panel (A) shows the gastric acid output for 10 min. Panel (B) shows the total acid output for 120 min. Each value and column represents the mean±S.E.M. for four to six rats. [a]$P<0.01$, significantly different from the control group. [b]$P<0.05$, significantly different from the capsaicin-treated group without the antagonist.

treated rats. As described above, the i.c.v. injection of neurokinin A (10 nmol), [β-Ala[8]]neurokinin A-(4-10) (10 nmol), and human CGRP (30 nmol) had a limited or marginal stimulatory effect on gastric acid secretion, and the effects were dependent on the animals. Even in the rats responding to these agonists, the acid output returned to the baseline at 3 h after the injections. In all animals tested, gastric acid secretion induced by the i.c.v. injection of capsaicin (30 nmol) was significantly inhibited in the neurokinin A- and [β-Ala[8]]neurokinin A-(4–10)-treated groups (Table 1). Similarly, the capsaicin response was significantly inhibited in the CGRP-treated group. In the neurokinin A- and CGRP-treated groups, the time course pattern of gastric acid secretion induced by capsaicin was similar to that in the control group. The gastric acid secretion induced by NMDA

(68 nmol) in the [β-Ala^8]neurokinin A-(4–10)- and CGRP-treated groups was similar to that in the control group; the values for total acid output (μEq HCl for 60 min) were 40.2±11.6 (n=6) in the control group, 45.3±14.8 (n=5) in the [β-Ala^8]neurokinin A-(4–10)-treated group and 58.8±9.0 (n=3) in the CGRP-treated groups. In addition, the total acid output with kainate (0.5 nmol, i.c.v.) in the neurokinin A- and CGRP-treated groups was 170–230 μEq HCl per 120 min, which was similar to that in the control group as shown in Fig. 3.

4. Discussion

4.1. Stimulation of gastric acid secretion by tachykinin NK_2 receptor activation in the brain regions close to the lateral cerebroventricle in rats

The present experiments were performed in urethane-anesthetized rats. The i.c.v. injection of neurokinin A in the presence of a tachykinin NK_1 receptor antagonist (L-703606) and [β-Ala^8]neurokinin A-(4–10) stimulated gastric acid secretion, and the effect of neurokinin A plus L-703606 was inhibited by a tachykinin NK_2 receptor antagonist (L-659877). The secretion induced by neurokinin A plus L-703606 was inhibited by an antagonist of non-NMDA receptors (CNQX). In addition, gastric acid secretion induced by vanilloid VR1 receptor activation in the CNS was inhibited by an antagonist of tachykinin NK_2 receptors, but not of tachykinin NK_1 receptors. The mammalian tachykinin family and their receptors are expressed in various brain regions in mammals including the rat (Ding et al., 2000; Severini et al., 2002). Recent findings showed that tachykinins such as neurokinin A are contained in the hypothalamic neurons (De Laurentiis et al., 2003), and stimulation of the lateral hypothalamus produced antinociception via tachykinin NK receptors (Holden et al., 2002). The present findings and these previous observations suggest that stimulation of tachykinin NK_2 receptors in the brain region close to the lateral cerebroventricle regulates gastric acid secretion positively.

Improta et al. (1997) reported that the i.c.v. injection of [Ala^5]neurokinin A-(4–10) (5 μg (6.4 nmol) per rat), which is a selective agonist of tachykinin NK_2 receptors, inhibited gastric acid secretion in rats. The discrepancy between their and our findings may be derived from the difference between afferent and efferent neurons, because Improta et al. (1997) measured gastric acid secretion during 2-h pylorus ligation in rats, not with the perfusion methods used in the present study. Jocic et al. (2001) reported the involvement of tachykinin NK_1 and NK_2 receptors in the medullary transmission of vagal afferent input from the acid-threatened rat stomach caused by intragastric administration of 0.5 M HCl. Thus, tachykinin NK_2 receptors on the afferent and efferent pathways in the CNS may regulate gastric acid secretion negatively and positively, respectively. Otherwise, the discrepancy may be explained by the difference in the animal's condition, urethane-anesthetized vs. conscious rat.

Okuma and Osumi (1991) reported that intrathecal administration of substance P (1–10 nmol per rat) inhibited gastric acid secretion via inhibition of the sympatho-adrenomedullary system in the preoptic area. Yang and Taché (1997) also reported that the unilateral microinjection of a stable substance P analog (50 and 100 nmol) into the dorsal vagal complex inhibited medullary thyrotropin-releasing hormone-induced gastric acid secretion. In accordance with these findings, the activation of tachykinin NK_1 receptors in the brain regions close to the lateral cerebroventricle is likely to inhibit gastric acid secretion, because the stimulatory effect of neurokinin A was observed constantly in the presence of a tachykinin NK_1 receptor antagonist in the present study (Fig. 1). The inhibitory pathway mediated by tachykinin NK_1 receptor activation may explain an unstable response to neurokinin A alone.

4.2. Stimulation of gastric acid secretion by CGRP receptor activation

The calcitonin family of peptides including CGRP and their receptor components such as the calcitonin-like receptor and the receptor activity-modifying proteins (RAMP1, 2 and 3) are expressed in various brain regions including the hypothalamus, and the i.c.v. injection of CGRP showed

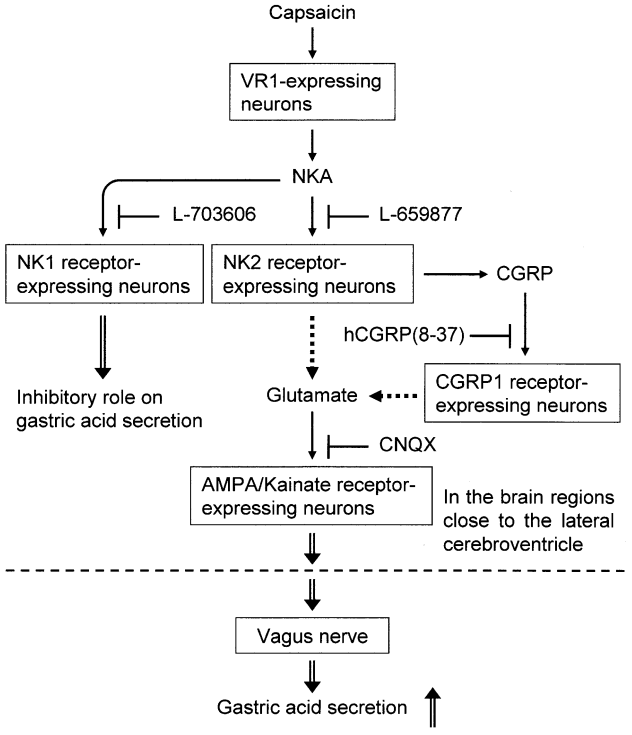

Fig. 5. Interactions between vanilloid VR1 receptor-, tachykinin NK_1 receptor-, CGRP1 receptor- and non-NMDA receptor systems regulating gastric acid secretion in the brain regions close to the lateral cerebroventricle in rat. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

various pharmacological effects in rats (Van Rossum et al., 1997; Kobayashi et al., 1999; Oliver et al., 2001; Poyner et al., 2002). A number of previous studies had shown that i.c.v. or intrahypothalamic injection of CGRP inhibited gastric acid secretion in conscious rats (Taché et al., 1984; Lenz et al., 1984, 1985a,b, 1989). In the present study, the i.c.v. injection of CGRP alone showed a limited effect on gastric acid secretion, but the secretions induced by vanilloid VR1 receptors and tachykinin NK_2 receptor activation were inhibited by the i.c.v. injection of hCGRP-(8–37), an antagonist of CGRP1 receptor. Our findings suggest that the efferent neurons having vanilloid VR1 receptors and/or tachykinin NK_2 receptors in the brain regions close to the lateral cerebroventricle stimulate the neurons having CGRP and/or CGRP1 receptors, and thus stimulate gastric acid secretion in urethane-anesthetized rats. A possible reason for the difference between the former and our findings may be derived from the CGRP receptor subtypes. The receptors for CGRPs have been divided into various classes. The functional CGRP1 receptors are composed of the calcitonin-like receptor and the RAMP1, although the molecular compositions of receptors for other peptides such as calcitonin and amylin are different (Poyner et al., 2002). Guidobono et al. (1994) reported that the i.c.v. injection of amylin decreased gastric acid secretion in rats. The subtypes of CGRP receptors regulating gastric acid secretion in various nuclei in the hypothalamic regions should be studied in the future. In addition, it is reported that urethane treatment had a profound effect on gastric acid secretion in rats through the release of somatostatin in the periphery (Kawakubo et al., 1999). The roles of CGRPs and its receptors in the CNS in gastric acid secretion should be studied in conscious freely moving rats.

4.3. Interaction between vanilloid VR1, tachykinin NK_2, CGRP1 and non-NMDA receptor systems in the brain regions close to the lateral cerebroventricle in rats

A possible mechanism for the vanilloid VR1 receptor-mediated response is shown in Fig. 5. The secretion induced by vanilloid VR1 receptor activation was inhibited by the i.c.v. injection of a selective antagonist of tachykinin NK_2 receptors (Fig. 1) and by the i.c.v. injection of CNQX (manuscript in preparation). Since tachykinin NK_2 receptor-mediated gastric acid secretion was almost completely inhibited by the i.c.v. injection of CNQX, and the effect of kainate was not inhibited by the antagonist of tachykinin NK_2 receptor, tachykinin NK_2 receptor-expressing neurons appear to exist upstream of the glutamate receptor-expressing neurons. Thus, vanilloid VR1 receptor activation appears to stimulate the tachykinin NK_2 receptor system and the next non-NMDA receptor system in the brain, and then stimulate gastric acid secretion via the vagus cholinergic nerve in rats (Minowa et al., 2001). In the rat hypothalamus, capsaicin evoked glutamate release from slices (Sasamura et al., 1998) and glutamate-immunopositive neurons expressed tachykinin NK receptor mRNA (Yao et al., 2001). The gastric acid secretions induced by activation of vanilloid VR1 receptors and tachykinin NK_2 receptors were inhibited by the antagonist of CGRP1 receptors. In addition, gastric acid secretion induced by capsaicin was decreased by pretreatment with neurokinin A, [β-Ala^8]neurokinin A-(4–10) and CGRP. These findings suggest a cross-interaction between vanilloid VR1-, tachykinin NK_2 receptors- and CGRP1 receptor-expressing neurons in the brain regions close to the lateral cerebroventricle in rats. Further neurochemical and/or histological studies of the interaction between the vanilloid VR1, tachykinin NK_2, CGRP1 and non-NMDA receptor systems are needed.

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