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Gastric acid secretion stimulated by centrally injected nociceptin in urethane-anesthetized rats

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Abstract

Nociceptin is a preferred endogenous ligand for the orphan opioid receptor-like 1 (ORL1) receptor. Central administration of nociceptin showed various pharmacological effects on analgesia, cardiovascular and renal responses, food intake, and so on. In the present study, we investigated the effect of nociceptin injected into the central nervous system (CNS) on gastric acid secretion in the perfused stomach of urethane-anesthetized rats. Injection of nociceptin (0.55–5.52 nmol per rat) into the fourth cerebroventricle stimulated gastric acid secretion and the secretion was inhibited in atropine-treated (1 mg/kg, i.v.) and vagotomized rats. The secretion induced by nociceptin (1.65 nmol) was not inhibited by the central injection of naloxone (275 nmol, a non-selective antagonist of opioid receptors). The secretion was significantly inhibited by the central injection of [Phe$^1\psi$(CH_2-NH)Gly^2]nociceptin-(1–13)-NH_2 ([F/G]nociceptin-(1–13), 0.21 nmol, an antagonist of ORL1 receptor), although [F/G]nociceptin-(1–13) alone at higher doses (2.10 and 7.31 nmol) markedly stimulated gastric acid secretion. In the 0–40 min period, the secretion induced by nociceptin was inhibited at least partially by CompB (68.8 nmol, a nonpeptidic antagonist of ORL1 receptor). Injection of nociceptin (5.52 nmol) into the lateral cerebroventricle also stimulated the secretion. Injection of nociceptin did not modify gastric acid secretion stimulated by 2-deoxy-D-glucose (200 mg/kg, i.v.). In conclusion, nociceptin injected into the CNS stimulated gastric acid secretion in rats via the ORL1 receptors and through mechanisms involving the vagus nerve. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Gastric acid secretion; Nociceptin; ORL1 receptor; Central injection; (Rat)

1. Introduction

Nociceptin is a 17 amino acid peptide, structurally similar to dynorphin A-(1–17) and is the endogenous ligand for the opioid receptor-like 1 (ORL1) receptor that shares significant homology with classical opioid receptors (Reinscheid et al., 1995; Darland et al., 1998; Calò et al., 2000). Despite these similarities, the nociceptin/ORL1 receptor system and dynorphin A/κ-opioid receptor system do not have a pharmacological interaction (Mollereau et al., 1999). ORL1 receptors are widely distributed in the brain (Anton et al., 1996; Neal et al., 1999; Mollereau and Mouledous, 2000; Florin et al., 2000). The nociceptin/ORL1 receptor system in the central nervous system (CNS) has been reported to play an important role in the regulation of physiological functions such as pain perception, locomotor activity and food intake via respective anatomic and signal-transduction pathways (for review, see Calò et al., 2000; Polidori et al., 2000; Krowicki et al., 2000).

In the CNS, the dorsal vagal complex, consisting of the nucleus of the solitary tract and the dorsal motor nucleus, is integral to the regulation of gastrointestinal functions (Berthoud et al., 1991). It has been established that the dorsal vagal complex is a pivotal region in the central regulation of gastric acid secretion, and that various neuropeptides, including thyrotropin releasing hormone and neuropeptide Y in the dorsal vagal complex, regulate gastric acid secretion (Taché and Yang, 1990; Yang et al., 1993, 2000; McTigue and Rogers, 1995; Geoghegan and Pappas, 1997). Previously, we reported that central injection of κ-opioid receptor agonists including dynorphin A-(1–17) (Ishihara et al., 2001a,b) and an agonist of the vanilloid receptor (capsaicin, Minowa et al., 2001) into the fourth cerebroventricle stimulated gastric acid secretion in rats.

Nociceptin acts in the CNS to regulate gastrointestinal functions. For example, injection of nociceptin into the dorsal vagal complex increased intragastric pressure and gastric motility in rats (Krowicki et al., 1997), and injection of nociceptin into the intracerebroventricle (lateral cerebroventricle) inhibited gastrointestinal transit of a non-absorbable

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marker in mice (Rossi et al., 1998; Osinski et al., 1999; Osinski and Brown, 2000). High to moderate expression of the ORL1 receptor protein and mRNA has been observed in many brainstem areas including the dorsal vagal complex (Anton et al., 1996; Neal et al., 1999; Mollereau and Mouledous, 2000; Florin et al., 2000). However, the effect of nociceptin in the CNS on gastric acid secretion has not been established. In the present study, we investigated the effect of nociceptin injected into the CNS on gastric acid secretion in urethane-anesthetized rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (Takasugi Exp. Animals, Kasukabe, Japan) weighing 210–320 g were used. The animals were housed under controlled environmental conditions (kept at 24 ± 2 °C with lights on 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

Nociceptin (human) and nocistatin (human) were purchased from PEPTIDE Institute (Osaka, Japan). [Phe^{1}ψ(CH_2-NH)Gly^2]nociceptin-(1–13)-NH_2 ([F/G]nociceptin-(1–13)) and naloxone hydrochloride were obtained from Sigma (St. Louis, MO, USA). CompB (alias J113397, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one]) was from Banyu Pharm. (Tsukuba, Japan). Atropine sulfate and 2-deoxy-D-glucose were obtained from Wako (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. The doses of the drugs used for central injection per rat were as follows: nociceptin (0.55–5.52 nmol (1–10 μg) per rat), naloxone (275 nmol (100 μg)), [F/G]nociceptin-(1–13) (0.21–7.31 nmol (0.3–10 μg)), CompB (22.9 and 68.8 nmol (10 and 30 μg)) and nocistatin (0.84 and 2.81 nmol (3 and 10 μg)). All compounds were dissolved in 0.9% saline, and the volume for central injections was 5 μl. 2-Deoxy-D-glucose (200 mg/kg) and atropine sulfate (1 mg/kg) were administered through an intravenous cannula inserted into the femoral vein.

2.3. Cannulation for central injection

Rats were anesthetized with urethane (1.35 g/kg, i.p.). The rats were placed on a stereotaxic instrument (SR-6, Narishige Scientific Instrument Lab., Tokyo, Japan), and a 24-gauge stainless steel guide cannula for 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. For the injection into the fourth cerebroventricle, the implanting coordinates were 11.5 mm posterior to the bregma, 0.0 mm lateral and 7.5 mm vertical from the surface of the skull. The cannula was secured with dental cement. At the end of the experiments, Evans blue solution was injected to confirm that the solution had diffused into each cerebral cavity. The femoral vein was cannulated for intravenous administration. To investigate the involvement of the vagus nerve in the mechanism of the effect of nociceptin, rats underwent bilateral vagotomy at the cervical level or sham operation after the implantation of cannulas.

2.4. Experimental procedures and measurement of gastric acid secretion

After the cannulation for injections, the animals were operated on for the measurement of gastric acid secretion. The rats were used for the measurement of secretion 1 h after the implantation of cannulas and the bilateral vagotomy. After the determination of basal acid secretion for 30 min, each test compound was injected. Antagonists were given 10 min before the injection of nociceptin. Gastric acid secretion was determined by gastric perfusion methods as described previously (Watanabe et al., 2000; Ishihara et al., 2001a,b; Tsuchiya et al., 2001). 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) through the inlet tube of the dual cannula connected to the perfusion pump at the rate of 1 ml/min. The stomach was maintained at a pressure of 5 cm H_2O. After 30 min of pre-perfusion, each test compound was injected. For observation of the effect of nociceptin on 2-deoxy-D-glucose-stimulated gastric acid secretion, nociceptin was administered 10 min before i.v. injection of 2-deoxy-D-glucose. Atropine sulfate and naloxone were administered 10 and 20 min before injection of nociceptin, respectively. 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 using an autonomic titrator (AUT-201, Toa Electronics, Japan). Under these conditions, titration to pH 5.0 was used to avoid the buffering action of gastric mucus. The acid output was expressed in terms of μEq HCl/10 min. In some experiments, values were presented as the total acid output for 40 or 90 min to show the results more clearly.

2.5. Statistical analysis

The values are expressed as mean ± S.E.M. for the indicated number of rats. The statistical significance of differ-

ences between two groups was assessed with Student's *t*-test followed by the *F*-test. In the case of multiple comparisons, the significance of differences was determined using a one-way analysis of variance followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of central injection of nociceptin, an endogenous agonist for the ORL1 receptor, into the fourth cerebroventricle on gastric acid secretion

The injection of nociceptin into the fourth cerebroventricle increased gastric acid secretion in a dose-dependent manner (Fig. 1). Gastric acid secretion began to increase about 20 min after the injection of nociceptin (1.65 and 5.52 nmol per rat), and gradually increased until the peak level was reached at 30–40 min. The secretion then returned to its baseline at 70–80 min. Regarding the total acid output for 90 min, a low dose (0.55 nmol) of nociceptin significantly increased gastric acid secretion (Fig. 1B). Next, to investigate whether the vagus nerve was involved in the mechanism of the stimulatory effect of nociceptin, we used two treatments; atropine administration and vagotomy (Fig. 2). Atropine sulfate (1 mg/kg. i.v.) was administered 10 min before the injection of nociceptin (1.65 nmol) into the fourth cerebroventricle. The gastric acid secretion stimulated by nociceptin was completely inhibited by atropine administration. In another experiment, a group of rats underwent bilateral vagotomy at the cervical level. The gastric acid secretion stimulated by nociceptin was also completely inhibited in the vagotomized rats. These findings suggest that the stimulatory effect of nociceptin injected into the fourth cerebroventricle on gastric acid secretion is mediated via the vagus cholinergic nerve.

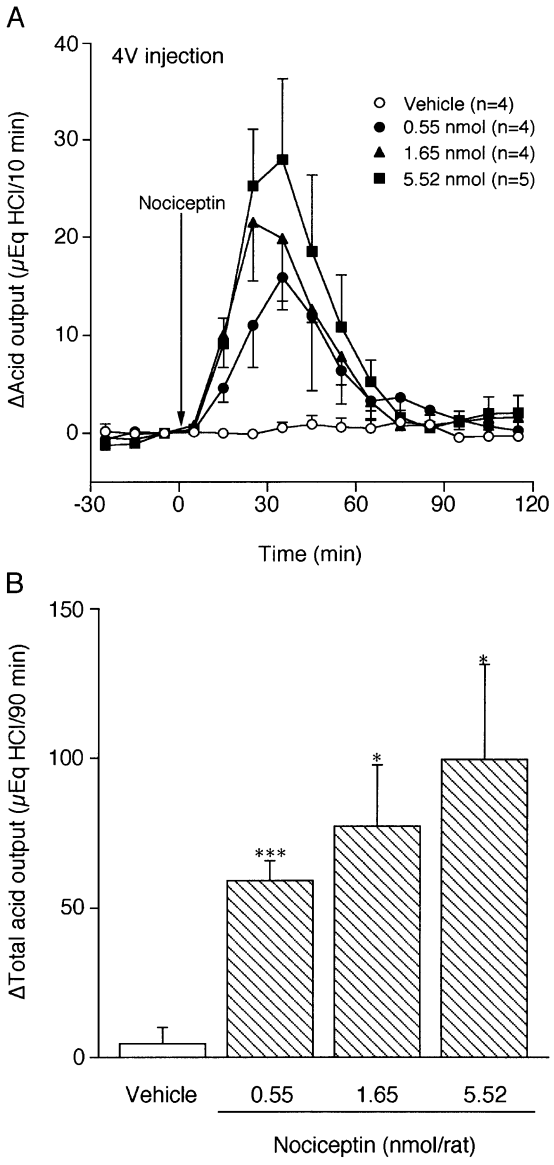

Fig. 1. Effect of nociceptin injected into the fourth cerebroventricle on gastric acid secretion. Vehicle (saline, 5 μl) or nociceptin (0.55, 1.65 and 5.52 nmol per rat, 5 μl) was injected into the fourth cerebroventricle (4V). (Panel A) Each value represents the gastric acid output for 10 min. (Panel B) The total gastric acid output for 90 min (in the period of 0–90 min) is shown. Each value and column represents the mean ± S.E.M. for four to five rats. $*P < 0.05$, $***P < 0.01$, statistically significant compared with the control (vehicle) groups.

3.2. Effects of nocistatin, naloxone and [F/G]nociceptin-(1–13) on nociceptin-stimulated gastric acid secretion

It was recently shown that nocistatin, a novel neuropeptide encoded by the gene for the nociceptin precursor, could reverse the effects induced by nociceptin (Okuda-Ashitaka et al., 1998; Nicol et al., 1998), although nocistatin did not bind with nociceptin-binding sites. Injection of nocistatin (0.84 and 2.81 nmol per rat) into the fourth cerebroventricle did not inhibit nociceptin (1.65 nmol)-stimulated gastric acid secretion; the total acid outputs for 90 min in the nociceptin-treated, 0.84 nmol nocistatin plus nociceptin-treated, and 2.81 nmol nocistatin plus nociceptin-treated groups were 72.8 ± 20.9 ($n = 4$), 79.9 ± 41.6 ($n = 5$) and 75.8 ± 26.2 μEq HCl ($n = 4$), respectively. Nocistatin alone had no effect on gastric acid secretion.

Fig. 3 shows the effect of naloxone, a non-selective antagonist for opioid receptors, on gastric acid secretion. The injection of naloxone (275 nmol per rat) into the fourth cerebroventricle did not block the stimulatory effect of nociceptin (1.65 nmol).

Fig. 4 shows the effect of [F/G]nociceptin-(1–13), an antagonist of the ORL1 receptor (Guerrini et al., 1998; Shah et al., 1998; Bigoni et al., 1999), on gastric acid secretion. The gastric acid secretion stimulated by nociceptin (1.65 nmol) was inhibited by the pre-injection of 0.21 nmol of [F/G]nociceptin-(1–13) into the fourth cerebroventricle (Panel A). Although significance was only reached for the acid output in the period of 20–30 min because of wide variation,

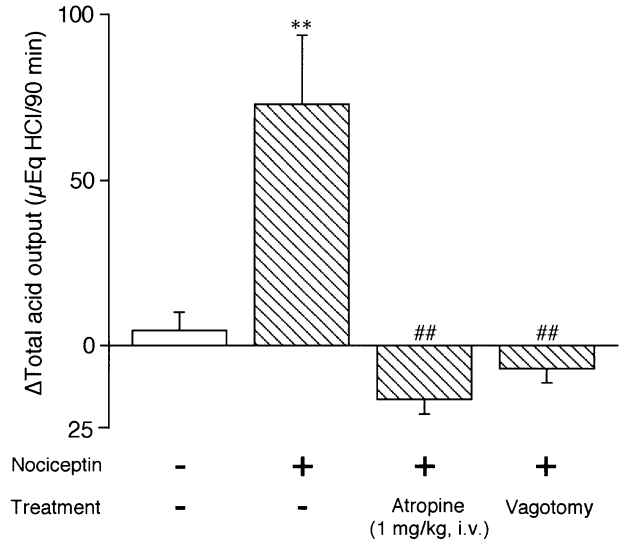

Fig. 2. Effects of atropine and vagotomy on gastric acid secretion stimulated by nociceptin injected into the fourth cerebroventricle. Atropine (1 mg/kg, i.v.) was injected 10 min before injection of nociceptin (1.65 nmol per rat) into the fourth cerebroventricle. Bilateral vagotomy at the cervical level or sham operation was performed 60 min before the experiment. The total gastric acid output for 90 min is shown. Each column represents the mean ± S.E.M. for four to five rats. The total gastric acid outputs without nociceptin in the atropine-treated and vagotomized-rats were similar to that in the control (non-treated) groups. $^{**}P<0.01$, statistically significant compared with the control (vehicle) groups. $^{\#\#}P<0.01$, statistically significant compared with the control groups injected with nociceptin.

[F/G]nociceptin-(1–13) tended to decrease the secretion for 40 min after the injection of nociceptin; the total acid outputs for 40 min in the nociceptin-treated and nociceptin plus [F/G]nociceptin-(1–13)-treated rats were 47.1 ± 9.6 ($n=7$) and 17.6 ± 8.1 μEq HCl ($n=5$, $P=0.051$, compared with the nociceptin-treated groups), respectively. [F/G]Nociceptin-(1–13) at this dose itself slightly stimulated secretion; the total acid outputs for 90 min in the 0.21 nmol [F/G]nociceptin-(1–13)-treated and the control groups were 20.2 ± 13.2 ($n=4$) and 1.1 ± 2.5 μEq HCl ($n=4$), respectively. Interestingly, 7.31 nmol of [F/G]nociceptin-(1–13) alone stimulated gastric acid secretion markedly (Fig. 4B); the total acid output for 90 min in the 7.31 nmol [F/G]nociceptin-(1–13)-treated groups was 177 ± 13 μEq HCl ($n=3$). The effect of 7.31 nmol of [F/G]nociceptin-(1–13) on gastric acid secretion continued for 120 min, and the effects of [F/G]nociceptin-(1–13) and nociceptin (1.65 nmol) were not additive. The injection of 2.1 nmol of [F/G]nociceptin-(1–13) also stimulated gastric acid secretion; the total acid outputs for 90 min in the 2.1 nmol [F/G]nociceptin-(1–13)-treated rats were 201 and 237 μEq HCl ($n=2$). In a typical experiment, the effect of 2.1 nmol of [F/G]nociceptin-(1–13) decreased about 80 min after the injection (data not shown). These findings suggest that the effect of centrally injected nociceptin on gastric acid secretion is mediated via [F/G]nociceptin-(1–13)-sensitive ORL1 receptors in the CNS.

3.3. Effect of CompB on nociceptin-stimulated gastric acid secretion

Next, we investigated the effect of CompB (alias J113397), a nonpeptidic ORL1 receptor antagonist (Ozaki et al., 2000; Corboz et al., 2000), on gastric acid secretion. Injection of CompB (68.8 nmol) alone into the fourth cerebroventricle stimulated the secretion 30–40 min after the injection, with a gradual increase until the peak level was reached at 50–60 min, and the secretion continued for 120 min (Fig. 5A). During the initial phase (0–40 min after the injection of nociceptin), however, CompB tended to inhibit gastric acid secretion induced by nociceptin (1.65 nmol). The total acid output in the 0–40 min period stimulated by nociceptin (1.65 nmol) in the CompB-treated rats was 24.7 ± 8.3 μEq HCl/40 min ($n=5$), which was low ($P=0.114$, not significant) compared with that in control rats (nociceptin alone, 51.0 ± 10.2 μEq HCl/40 min, $n=4$). The total acid output in the CompB-treated rats without nociceptin was 20.8 ± 9.3 μEq HCl/40 min ($n=6$). Injection of a low dose (22.9 nmol) of CompB alone also stimulated gastric acid secretion and did not inhibit the secretion stimulated by a low dose (0.55 nmol) of nociceptin (Fig. 5B). Interestingly, in the period of 60–120 min, the gastric acid secretion induced by CompB (22.9 and 68.8 nmol) appeared to be inhibited in the nociceptin-injected rats. These findings suggest that the effect of centrally injected nociceptin on gastric acid secretion was modified at least partially by CompB. The reason(s) why the centrally in-

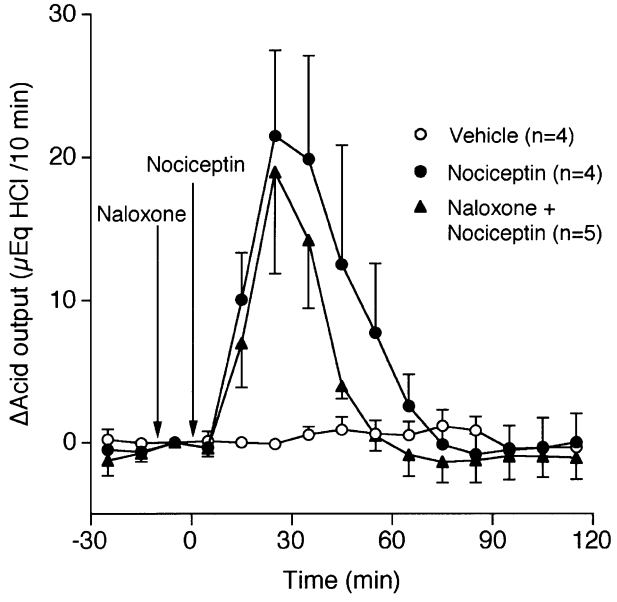

Fig. 3. Effect of naloxone, a general antagonist for opioid receptors, on gastric acid secretion stimulated by nociceptin injected into the fourth cerebroventricle. Vehicle or naloxone (275 nmol per rat) was injected into the fourth cerebroventricle 10 min before the injection of 1.65 nmol of nociceptin. Each value represents the gastric acid output for 10 min and the mean ± S.E.M. for four to five rats.

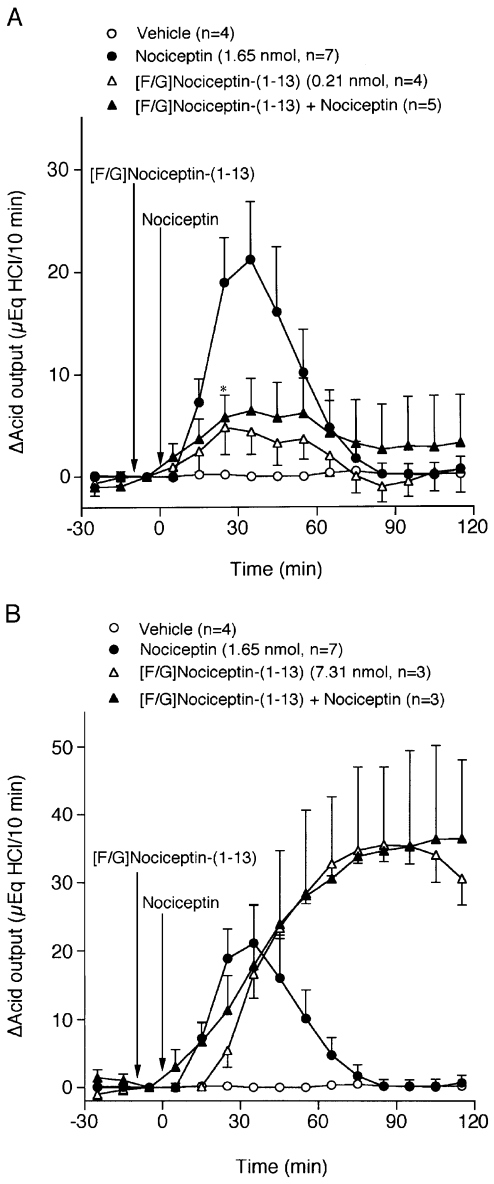

Fig. 4. Effect of [F/G]nociceptin-(1–13) on gastric acid secretion. (Panel A) Inhibition of nociceptin-stimulated gastric acid secretion by a low dose of [F/G]nociceptin-(1–13) injected into the fourth cerebroventricle. Vehicle or [F/G]nociceptin-(1–13) (0.21 nmol per rat) was injected into the fourth cerebroventricle 10 min before the injection of nociceptin (1.65 nmol). (Panel B) Stimulatory effect of a high dose of [F/G]nociceptin-(1–13) on gastric acid secretion. Vehicle or [F/G]nociceptin-(1–13) (7.31 nmol per rat) was injected into the fourth cerebroventricle 10 min before the injection of nociceptin (1.65 nmol). Each value represents the mean ± S.E.M. for three to seven rats. $*P<0.05$, statistically significant compared with the control (vehicle) groups injected with nociceptin.

jected CompB alone stimulated secretion was (were) not clarified.

3.4. Effect of nociceptin on 2-deoxy-D-glucose-stimulated gastric acid secretion

We investigated the effect of nociceptin on 2-deoxy-D-glucose-stimulated secretion (Fig. 6). Injection of nociceptin (1.65 nmol) into the fourth cerebroventricle slightly enhanced the secretion in the 0–30 min after the i.v. injection of 2-deoxy-D-glucose, probably due to the stimulatory effect of nociceptin. Nociceptin did not inhibit the 2-deoxy-D-

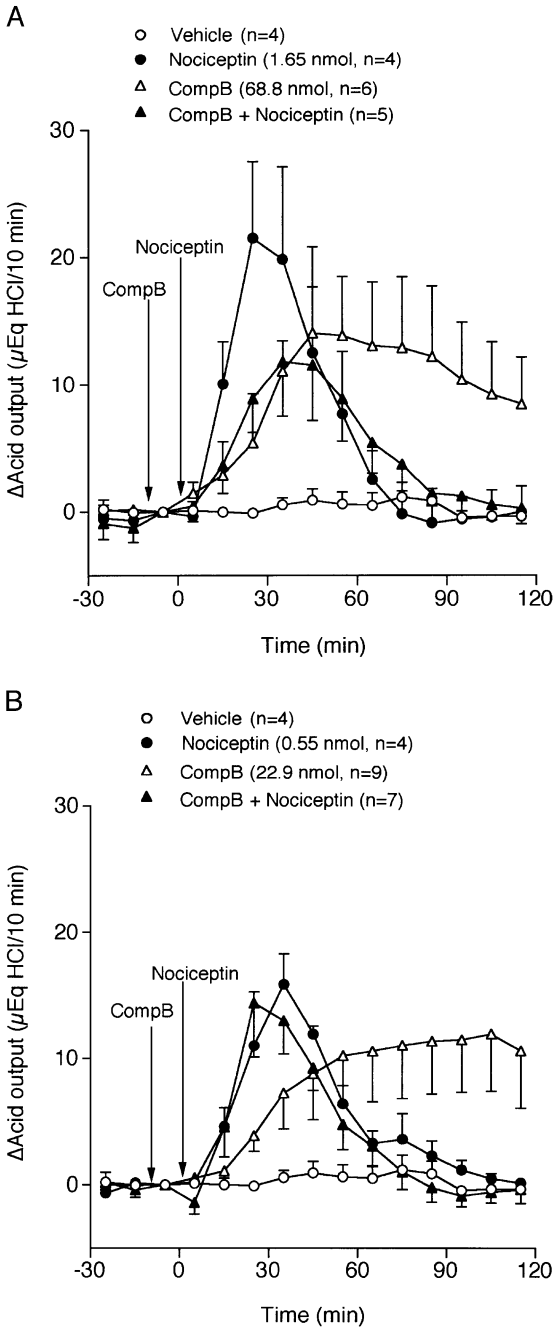

Fig. 5. Effect of CompB injected into the fourth cerebroventricle on nociceptin-stimulated gastric acid secretion. Vehicle or CompB (68.8 nmol (Panel A), 22.9 nmol (Panel B) per rat) was injected into the fourth cerebroventricle 10 min before the injection of nociceptin (1.65 nmol (Panel A), 0.55 nmol (Panel B) per rat). Each value represents the gastric acid output for 10 min and represents the mean ± S.E.M. for four to nine rats. In Panel A, the differences in P values at 15 and 25 min between the nociceptin-treated groups and the nociceptin/CompB-treated groups were 0.163 and 0.123, respectively.

glucose-stimulated secretion. Nociceptin, even at low doses (1.65 pmol), did not show an inhibitory effect on the 2-deoxy-D-glucose-stimulated secretion.

3.5. Effect of nociceptin injected into the lateral cerebroventricle on gastric acid secretion

Next, we investigated the effect of nociceptin injected into the lateral cerebroventricle. As shown in Fig. 7A, gastric acid secretion induced by nociceptin (5.52 nmol) injected into the lateral cerebroventricle showed a pattern similar to that with nociceptin injected into the fourth cerebroventricle: the secretion increased 20 min after the injection, reached the maximum level after 40 min, and returned to the baseline at 90 min. However, injection of 0.55 and 1.65 nmol of nociceptin into the lateral cerebroventricle, a dose which significantly stimulated the secretion on injection into the fourth cerebroventricle, did not stimulate gastric acid secretion.

3.6. Effect of subcutaneous injection of naloxone on gastric acid secretion induced by injection of nociceptin into the fourth cerebroventricle

Food intake stimulated by injection of nociceptin into the lateral cerebroventricle (Pomonis et al., 1996) and into the ventromedial hypothalamic nucleus or the nucleus accumbens shell (Stratford et al., 1997) were reversed by subcutaneous injection of naloxone (0.3 mg/kg) in rats. However, the gastric acid secretion stimulated by nociceptin injected into the fourth cerebroventricle was not inhibited by subcutaneous injection of naloxone (1 mg/kg) (Fig. 7B).

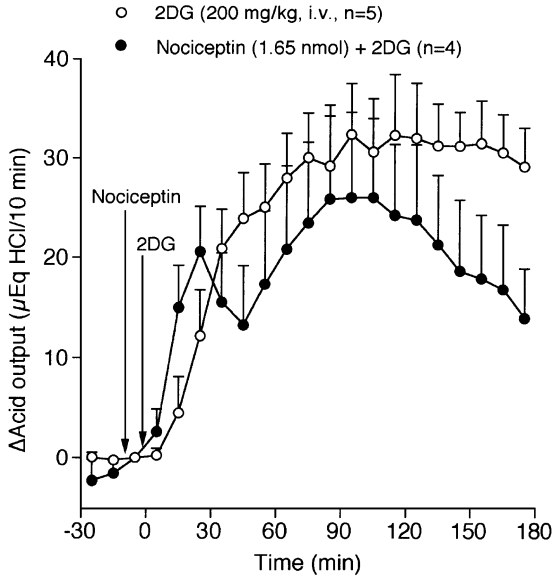

Fig. 6. Effect of nociceptin injected into the fourth cerebroventricle on gastric acid secretion stimulated by 2-deoxy-D-glucose. Vehicle or nociceptin (1.65 nmol per rat) was injected into the fourth cerebroventricle 10 min before the injection of 2-deoxy-D-glucose (200 mg/kg, i.v.). Each value represents the gastric acid output for 10 min and represents the mean ± S.E.M. for four to five rats.

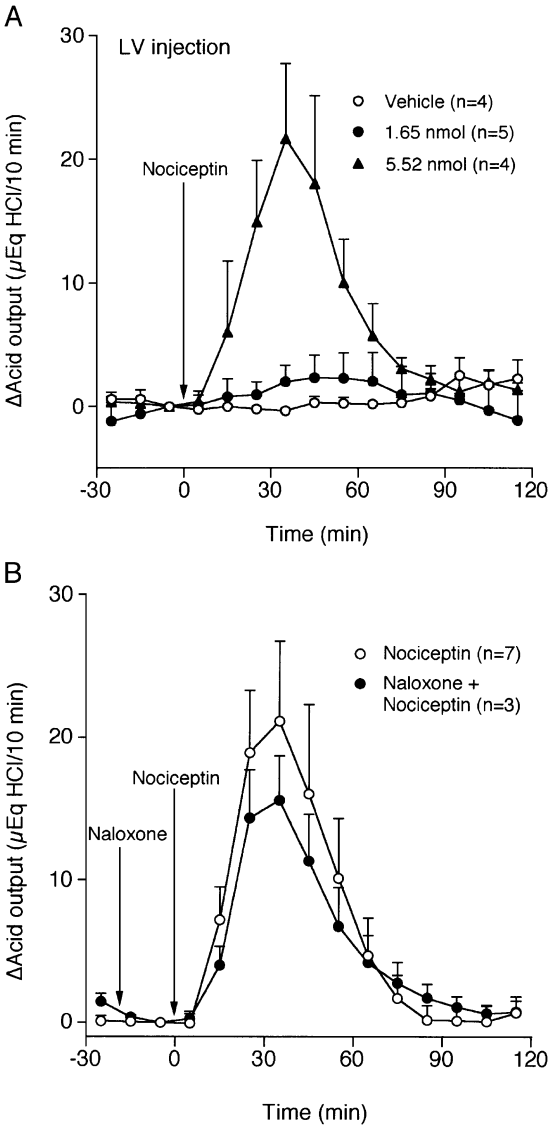

Fig. 7. (Panel A) Effect of nociceptin injected into the lateral cerebroventricle on gastric acid secretion. Vehicle or nociceptin (1.65 and 5.52 nmol per rat) was injected into the lateral cerebroventricle (LV). (Panel B) Effect of subcutaneous injection of naloxone on nociceptin-stimulated gastric acid secretion. Vehicle (saline) or naloxone (1 mg/kg, s.c.) was administered 20 min before injection of nociceptin (5.52 nmol per rat) into the fourth cerebroventricle. Each value represents the gastric acid output for 10 min and represents the mean ± S.E.M. for three to seven rats.

4. Discussion

4.1. Stimulation of gastric acid secretion by nociceptin injected into the CNS in rats

As mentioned in the Introduction, nociceptin injected into the CNS regulates gastric functions such as gastric

contractile activity and intragastric pressure (Krowicki et al., 1997; Rossi et al., 1998; Osinski et al., 1999; Osinski and Brown, 2000). In addition, injection of nociceptin into the lateral or the third cerebroventricle (Pomonis et al., 1996; Polidori et al., 2000; Olszewski et al., 2000) and into the ventromedial hypothalamus or the shell of the nucleus accumbens (Stratford et al., 1997) markedly increases food intake in rats. Injection of nociceptin (2 μg (1.1 nmol) per rat) into the intracerebroventricle completely abolishes the hypophagic effect induced by stress or by injection of corticotropin releasing factor, but does not modify food consumption in food-deprived rats (Ciccocioppo et al., 2001). These findings suggest that nociceptin in the CNS has pharmacological and physiological roles in gastric functions and food intake. However, the effect of nociceptin in the CNS on gastric acid secretion has not been determined. In the present study, we investigated the effect of centrally injected nociceptin on gastric acid secretion in rats. Injection of nociceptin into the fourth (Fig. 1) and the lateral (Fig. 7A) cerebroventricle stimulated gastric acid secretion markedly. The effect of nociceptin was seen with a low dose (0.55 nmol) injected into the fourth cerebroventricle. Since the effect of nociceptin was not inhibited by naloxone injected into the fourth cerebroventricle (Fig. 3), the effect of nociceptin was not derived from the activation of opioid receptors in the CNS. The effect of centrally injected nociceptin was abolished by vagotomy and by atropine administration (Fig. 2). Previously, we reported that injection of morphine, a μ-opioid receptor agonist, into the fourth cerebroventricle inhibited gastric acid secretion stimulated by 2-deoxy-D-glucose (200 mg/kg, i.v.) (Ishihara et al., 2001a). However, nociceptin did not show an inhibitory effect on secretion (Fig. 6). These findings suggest that nociceptin injected into the CNS stimulated gastric acid secretion via the vagus cholinergic nerve in rats. The magnitude of the maximal gastric acid secretion induced by central injection of nociceptin in the present study was almost the same as that of secretion induced by other neuropeptides such as thyrotropin releasing hormone and dynorphin A-(1–17) (Taché and Yang, 1990; Yang et al., 1993, 2000; McTigue and Rogers, 1995; Ishihara et al., 2001b; Date et al., 2001). To our knowledge, this is the first study demonstrating that nociceptin injected into the CNS stimulates gastric acid secretion.

4.2. Involvement of ORL1 receptor in the CNS on gastric acid secretion stimulated by nociceptin

[F/G]Nociceptin-(1–13) has been reported to act as an antagonist of ORL1 receptors in membrane preparations obtained from mice and guinea pigs (Guerrini et al., 1998; Shah et al., 1998) and rats (Bigoni et al., 1999). The injection of [F/G]nociceptin-(1–13) (180 μg (131 nmol)) into the lateral cerebroventricle inhibits the antitussive effect of nociceptin (90 μg, 49.7 nmol) in guinea pigs (McLeod et al., 2001). Under the present study conditions, injection of [F/G]nociceptin-(1–13) (0.21 nmol) into the fourth cerebroventricle inhibited gastric acid secretion stimulated by 1.65 nmol of nociceptin (Fig. 4A). There are several reports that [F/G]nociceptin-(1–13) acts as an agonist in neuronal cells (Xu et al., 1998; Grisel et al., 1998) or in cells expressing the human ORL1 receptor (Butour et al., 1998; Okawa et al., 1999). Central injection of [F/G]nociceptin-(1–13) at 10 nmol per rat showed full agonistic activity for nociception (Candeletti et al., 2000), food intake (Polidori et al., 2000; Olszewski et al., 2000) and heart rate, mean arterial pressure, urinary sodium excretion and urine flow rate (Kapusta et al., 1999). Interestingly, [F/G]nociceptin-(1–13) at higher doses (2.10 and 7.31 nmol per rat) itself markedly stimulated gastric acid secretion, and the effect was not additive to the effect of nociceptin (Fig. 4B). Thus, [F/G]nociceptin-(1–13) injected into the fourth cerebroventricle appeared to act as an antagonist at low doses and as a full agonist at higher doses on the nociceptin-sensitive neurons causing gastric acid secretion in rats.

The nonpeptidic ORL1 receptor antagonist, CompB, has been shown to have a nanomolar potency at the ORL1 receptor with high selectivity over other opioid receptors (Ozaki et al., 2000; Corboz et al., 2000). Interestingly, the antagonistic effects of CompB in vivo on hyperalgesia (Ozaki et al., 2000) and antitussive activity (McLeod et al., 2001) elicited by central injection of nociceptin were seen with peripheral (s.c. or i.p.) injection of CompB, not with central injection of CompB. Although the effect of nociceptin injected into the fourth cerebroventricle was slightly inhibited by the central injection of CompB at a high dose (68.8 nmol), injection of CompB (22.9 and 68.8 nmol) into the fourth cerebroventricle alone stimulated gastric acid secretion markedly. Both CompB and [F/G]nociceptin-(1–13) showed slower starting (30–40 min after the injections) and longer lasting actions (continuation for 120 min) on gastric acid secretion (Figs. 4B and 5), compared with the effect induced by nociceptin. CompB, specifically in the CNS, may act as an agonist of the ORL1 receptor, probably like [F/G]nociceptin-(1–13). However, the effect of nociceptin injected into the fourth cerebroventricle was inhibited, at least partially, by the central injection of [F/G]nociceptin-(1–13) and CompB in rats in the present study.

Although the reasons for the discrepancy concerning the effects of [F/G]nociceptin-(1–13) and CompB are unknown, Xu et al. (1998) suggested that [F/G]nociceptin-(1–13) is more stable and/or resistant to degradation by aminopeptidases than the native nociceptin. Krowicki et al. (2000) suggested that the antagonistic properties of [F/G]nociceptin-(1–13) may be due to tachyphylaxis rather than to blockade of the ORL1 receptor, and factors such as the tissue ORL1 receptor reserve may determine the extent to which [F/G]nociceptin-(1–13) has an antagonistic role. Ueda et al. (2000) reported that CompB (J113397) blocked the anti-opioid nociceptin/ORL1 mechanism at the spinal rather than at the supraspinal level. Grond et al. (2001) proposed that the pro- and anti-inflammatory effects of

nociceptin might be mediated via different subtypes of the ORL1 receptor. We could not exclude the possibility of another type of nociceptin- ([F/G]nociceptin-(1–13)- and CompB-) binding site in addition to ORL1 receptor in the CNS, as reported previously (Xie et al., 2000).

4.3. Nociceptin-sensitive neurons regulating gastric acid secretion in the CNS

It was reported that ORL1 receptor mRNA and the radioligand binding sites are widely expressed in the rat CNS, such as in the forebrain, including hypothalamus and ventromedial nucleus, and in the brainstem, including the nucleus of the solitary tract and medial vestibular nucleus (Neal et al., 1999; Mollereau and Mouledous, 2000). These findings suggest that the stimulatory effect of nociceptin injected into the CNS on gastric acid secretion is mediated at least partially via the activation of ORL1 receptors on neurons in these areas. The effect of nociceptin injected into the fourth cerebroventricle was more potent than that of injection into the lateral cerebroventricle (Figs. 1 and 7A). Although the drugs administered by injection into the fourth cerebroventricle spread only around the brainstem, the drugs injected into the lateral cerebroventricle spread widely around the forebrain and the caudal regions such as the fourth cerebroventricle. As described in the Introduction, the dorsal vagal complex is a pivotal region for the central regulation of gastric acid secretion by neuropeptides (Taché and Yang, 1990; Yang et al., 1993, 2000; McTigue and Rogers, 1995; Geoghegan and Pappas, 1997). The dorsal vagal complex appeared to be a candidate target of nociceptin.

4.4. Problems to be solved and summary

Previously, we reported that the central injection of naloxone (82.5 nmol per rat) completely inhibited gastric acid secretion stimulated by central injection of κ_1-opioid receptor agonists such as (5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide (U59593) (Ishihara et al., 2001a). On the other hand, the gastric acid secretion stimulated by nociceptin injected into the fourth cerebroventricle was not inhibited by naloxone (275 nmol per rat) injected into the fourth cerebroventricle (Fig. 3), and not inhibited by subcutaneous injection of naloxone (1 mg/kg) (Fig. 7B). Recently, Chen et al. (2001) reported that the hypothermia induced by injection of nociceptin (9 μg, 5 nmol) into the intracerebroventricle was not inhibited by subcutaneous injection of naloxone (10 mg/kg). However, the increase in food intake on injection of nociceptin into the CNS was reversed by subcutaneous injection of naloxone (0.3 mg/kg) in rats (Pomonis et al., 1996; Stratford et al., 1997). The increase in food intake and that in c-*fos* immunoreactivity in several feeding-related brain sites induced by central injection of [F/G]nociceptin-(1–13) were inhibited by naloxone (0.3 mg/kg) (Olszewski et al., 2000). The dopamine release induced by nociceptin from the striatum of conscious freely moving rats was attenuated by naloxone (Konya et al., 1998). Since naloxone shows very little affinity for the ORL1 receptor and does not block the activity of nociceptin directly (Mollereau et al., 1999; Calò et al., 2000), the basis for such an interaction between the nociceptin/ORL1 receptor system and naloxone is not fully understood. As proposed (Calò et al., 2000; Olszewski et al., 2000), naloxone-sensitive mechanism(s), interneurons and/or the cross talk with the opioid receptor system may be involved in the nociceptin/ORL1 receptor system in the CNS.

It was shown that nociceptin regulates neurotransmitter release in the CNS (for review, see Schlicker and Morari, 2000). For example, nociceptin regulates the release of glutamate, 5-hydroxytryptamine and γ-aminobutyric acid in the CNS (Okawa et al., 1999; Meis and Pape, 2001). These neurotransmitters in the CNS have been shown to regulate gastric acid secretion (Geoghegan and Pappas, 1997). Previously, we reported that glutamate injected into the CNS stimulated gastric acid secretion via *N*-methyl-D-aspartate receptors (Tsuchiya et al., 2001) and that neuroactive progesterone metabolites stimulated the secretion via the A-type γ-aminobutyric acid receptor (Watanabe et al., 2000). The putative intertransmitter(s) effect on nociceptin response in the CNS remains to be determined.

In conclusion, central injection of nociceptin stimulated gastric acid secretion in the perfused stomach of urethane-anesthetized rats via ORL1 receptor and through vagus nerve mechanisms.

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