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European Journal of Pharmacology 418 (2001) 187–194

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Stimulatory effects of centrally injected κ-opioid receptor agonists on gastric acid secretion in urethane-anesthetized rats

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Received 30 November 2000; received in revised form 23 March 2001; accepted 28 March 2001

Abstract

Gastric acid secretion has been proposed to be regulated by opioid receptors in the central nervous system (CNS). However, whether the effect of morphine is stimulatory or inhibitory, and the role of type specificity of opioid receptors have not been established. We investigated the effects of centrally injected opioid receptor agonists on gastric acid secretion in the perfused stomach of urethane-anesthetized rats. Injection of morphine (1–30 μg/rat, μ-opioid receptor agonist) into the fourth cerebroventricle inhibited the secretion stimulated by i.v. injection of 2-deoxy-D-glucose. Morphine itself did not show an inhibitory effect. In contrast, 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, 0.3–3 μg) and (*trans*)-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl) benzeneacetamide hydrochloride (U50488H, 10 μg) and the κ_2-opioid receptor agonist, bremazocine (3 μg), into the lateral cerebroventricle markedly stimulated secretion. The effect of U59593 was inhibited by naloxone and norbinaltorphimine (an antagonist of κ-opioid receptors) and in vagotomized rats. [D-Pen^2-D-Pen^5]enkephalin (10 μg, δ-opioid receptor agonist) had no effect on secretion. The dual roles of the opioid system in the CNS in gastric acid secretion are discussed.

Keywords: Gastric acid secretion; Opioid receptor type; Central injection; (Rat)

1. Introduction

The recent development of studies related to endogenous opioids and opioid receptors have clarified the physiological roles of the opioid system in analgesia, locomotor activity and reward, etc. (for review, see McDowell and Kitchen, 1987; Zadina et al., 1999; Stefano et al., 2000). Opioid receptors are generally divided into three types, μ-, κ- and δ-opioid receptors (Zadina et al., 1999; Stefano et al., 2000). Each receptor type plays various important roles in both the peripheral and the central nervous system (CNS) (Satoh and Minami, 1995; Stefano et al., 2000). Gastric acid secretion is also regulated by the opioid system. It has been reported that i.p. injection of morphine, a μ-opioid receptor agonist, inhibited basal gastric acid secretion (Del Tacca et al., 1989) and the secretion stimulated by i.v. injection of 2-deoxy-D-glucose (Ho et al., 1987) in rats. Fox and Burks (1988) reported that i.v. injection of morphine inhibited the secretion in pylorus-ligated rats. We also reported that i.v. injection of morphine inhibited gastric acid secretion stimulated by 2-deoxy-D-glucose in the perfused stomach of urethane-anesthetized rats (Watanabe et al., 1987). In the CNS, the i.c.v. injection of morphine and β-endorphin (an endogenous μ-opioid receptor agonist) inhibited the secretion in rats with a gastric fistula (Roze et al., 1980), and morphine and Tyr-D-Ala-Gly-(NMe)Phe-Gly-ol (DAMGO, a peptidyl μ-opioid receptor agonist) inhibited gastric acid secretion in pylorus-ligated rats (Fox and Burks, 1988). These findings suggest an inhibitory effect of the μ-opioid receptor-mediated system on gastric acid secretion. However, several studies have shown that morphine stimulated gastric acid secretion under different conditions and in various animals (Konturek et al., 1980; Del Tacca et al., 1989), and the detailed site of the μ-opioid receptor agonist action in CNS has not been determined.

In addition, the κ-opioid receptor-mediated systems is suggested to have both stimulatory and inhibitory effects on gastric acid secretion. In pylorus-ligated rats, i.v. injection of (*trans*)-(±)-3,4-dichloro-*N*-methyl-*N*-(2-[1-pyrrolidinyl]cyclohexyl)benzeneacetamide hydrochloride (U50488H) or ethylketocyclazocine, κ-opioid receptor ag-

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)00949-9

onists, slightly stimulated gastric acid secretion (Fox and Burks, 1988). In the CNS, the i.c.v. injection of dynorphin (1–9) and ethylketocyclazocine stimulated the secretion slightly, but not significantly (Fox and Burks, 1988). However, Morley et al. (1981) reported that i.c.v. injection of dynorphin inhibited gastric acid secretion stimulated by central injection of thyrotropin-releasing hormone. Thus, whether activation of the κ-opioid receptor system stimulates or inhibits gastric acid secretion has not been determined in the CNS.

In the present study, we investigated the effects induced by the central injection of μ- and κ-opioid receptor agonists on gastric acid secretion in the perfused stomach of urethane-anesthetized rats. Central injection of κ-opioid receptor agonists such as (5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide (U69593) or U50488H significantly stimulated gastric acid secretion, but morphine (μ-opioid receptor agonist) inhibited 2-deoxy-D-glucose-stimulated secretion in rats. In addition, we compared the intensity of the effects of μ- and κ-opioid receptor agonists injected either in the fourth cerebroventricle or in the lateral cerebroventricle, in order to identify the site of the opioid effects. Our findings showed dual roles of the opioid system in the CNS in gastric acid secretion: inhibition by μ-opioid receptors and stimulation by κ-opioid receptors.

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 (temperature 24 ± 2°C and light between 7:00 AM and 7:00 PM) and fed commercial rat chows (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

Morphine hydrochloride was obtained from Takeda Chemical Industries, (Osaka, Japan). U69593, U50488H and (±)-6-ethyl-1,2,3,4,5,6-hexahydro-3-([1-hydroxycyclopropyl]methyl)-11,11-dimethyl-2,6-methano-3-benzazocin-8-ol hydrochloride (bremazocine) were obtained from RBI (Natic, MA, USA). [D-Pen^2-D-Pen^5]enkephalin (DPDPE) was obtained from BACHEM (Bubendorf, Switzerland). Norbinaltorphimine, naloxone hydrochloride and DAMGO were obtained from Sigma (St. Louis, MO, USA). 2-Deoxy-D-glucose and atropine sulfate were obtained from Nakarai Tesque (Kyoto, Japan) and Wako (Osaka, Japan), respectively. Urethane was obtained from Tokyo Kasei Kogyo (Tokyo, Japan).

U69593 was dissolved in a minimum of 0.1 N HCl and saline, the final pH was about 4.0. Morphine hydrochloride, DAMGO, U50488H, DPDPE, naloxone hydrochloride, norbinaltorphimine, 2-deoxy-D-glucose and atropine sulfate were dissolved in saline. Urethane was dissolved in distilled water. The doses of the drugs tested by central injection were chosen in accordance with previous reports (Roze et al., 1980; Konturek et al., 1980; Morley et al., 1981; Fox and Burks, 1988; Del Tacca et al., 1989) and the doses shown are per rat. Morphine (0.3–30 μg (0.43–43 nmol) per rat), DAMGO (1 μg (1.9 nmol)), U69593 (1–30 μg (2.8–84.1 nmol)), U50488H (10 μg (21.5 nmol)), DPDPE (10 μg (15.5 nmol)), naloxone (30 and 100 μg (82.5 and 275 nmol)), norbinaltorphimine (10 μg (12.6 nmol)) and bremazocine (3 μg (7.7 nmol)) were administered in a volume of 5 μl over 30 s using a microliter syringe through a guide cannula positioned in the brain. 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 or intravenous injection

The rats were anesthetized with urethane (1.35 g/kg, i.p.) and placed on a stereotaxic instrument (SR-6, Narishige Scientific Instrument Lab., Tokyo, Japan), and a 24-gauge stainless steel guide cannula for microinjection of drugs was implanted into the lateral cerebroventricle with the following coordinates taken from the atlas of Paxinos and Watson (1982): 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, implanting coordinates were as follows: 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 effects of κ-opioid receptor agonists, rats underwent bilateral vagotomy at the cervical level or sham operation after the implantation of cannulas.

2.4. Measurement of gastric acid secretion

The rats were used for the measurement of gastric acid secretion 1 h after the implantation of cannulas and the

vagotomy. This procedure is popular in research concerning short-term neuronal regulation of gastric acid secretion (Watanabe et al., 1987; Yang et al., 1993; Barrachina et al., 1995; García-Zaragozá et al., 2000). Gastric acid secretion was determined by the gastric perfusion method described by Watanabe et al. (1987, 2000) with minor modifications. The trachea was exposed, then cannulated and the esophagus was ligated at the cervical level. After laparotomy, the pylorus was ligated and a dual gastric 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 (Mini pump TMP-10H, Toyo Kagaku Sangyo, Japan) at rate of 1 ml/min. 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 injected. For observations of the inhibitory effect of opioid receptor agonists on 2-deoxy-D-glucose-stimulated gastric acid secretion, opioid receptor agonists were administered 10 min before i.v. injection of 2-deoxy-D-glucose. Opioid receptor antagonists and atropine sulfate were administered 10 min before opioid receptor agonist injection. The perfusate flowing from the outlet tube was collected as 10 min fractions with a fraction collector (Eyela DC-20, Tokyo Rikakikai, Japan) and titrated to pH 5.0 with 0.02 N NaOH using an autonomic titrator (AUT-201, Toa Electronics, Japan). Under our conditions, titration to pH 5.0 is used in order to avoid the buffering action of gastric mucus (Watanabe et al., 1987, 2000; Hasebe et al., 1998). The acid output was expressed in terms of μEq HCl/10 min. In some experiments, the total acid output for 90 or 120 min was measured.

2.5. Statistical analysis

The values are expressed as means ± S.E.M. for four to nine rats. The statistical significance of differences between two groups was assessed with Student's *t*-test followed by the *F*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of central injection of morphine, μ-opioid receptor agonist, on 2-deoxy-D-glucose-stimulated gastric acid secretion

2-Deoxy-D-glucose (200 mg/kg, i.v.) produced a sustained increase in gastric acid secretion, which reached a plateau at 1 h (Fig. 1A), as reported previously (Watanabe et al., 1987). Injection of 0.3 μg per rat of morphine into fourth cerebroventricle inhibited the secretion slightly, but not significantly. Injection of 3 or 30 μg of morphine significantly inhibited 2-deoxy-D-glucose-stimulated gastric acid secretion. The inhibitory effect of 3 μg of morphine continued over 2 h. The total acid output for 2 h after 2-deoxy-D-glucose administration is shown in Fig. 1B. Morphine significantly inhibited 2-deoxy-D-glucose-stimulated gastric secretion in a dose-dependent manner.

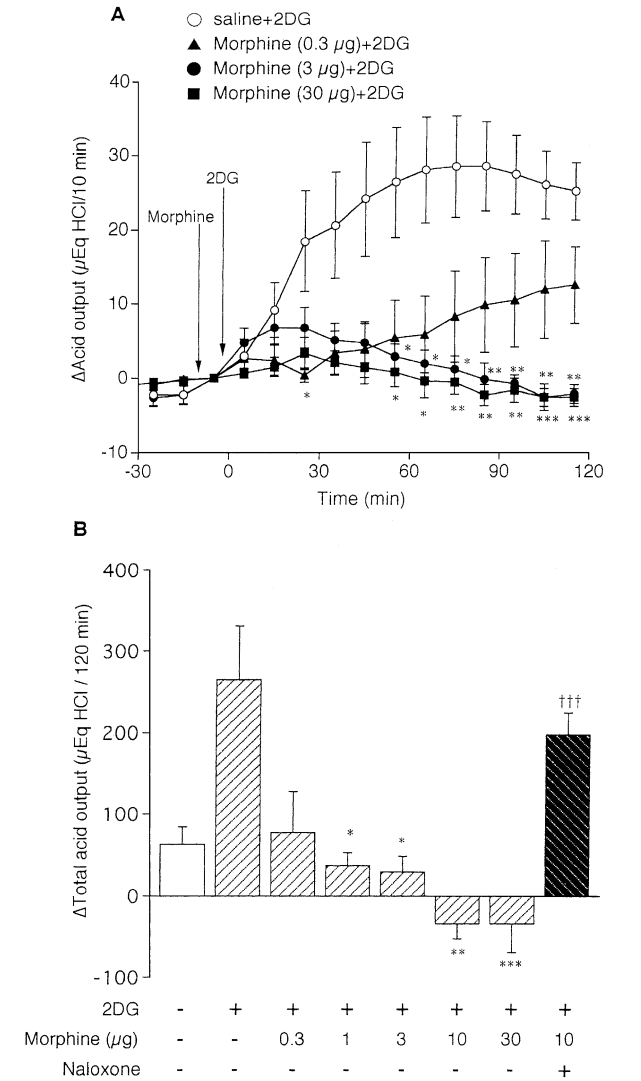

Fig. 1. Inhibitory effect of morphine injected into fourth cerebroventricle on gastric acid secretion stimulated by 2-deoxy-D-glucose in urethane-anesthetized rats. In panel A, vehicle (saline, 5 μl) or morphine (0.3, 3, 30 μg/rat) was microinjected into the fourth cerebroventricle 10 min before the injection of 2-deoxy-D-glucose (200 mg/kg, i.v.). Each value represents the gastric acid output for 10 min. The values are the means ± S.E.M. for four to nine rats. In panel B, the dose-dependent effect of morphine on the total gastric acid output for 120 min after the administration of 2-deoxy-D-glucose is shown. Naloxone (100 μg) was injected into fourth cerebroventricle 10 min before the fourth cerebroventricle injection of morphine (10 μg). Each column represents the mean ± S.E.M. for four to nine rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ statistically significant compared with the 2-deoxy-D-glucose-treated group. †††$P < 0.001$ compared with the morphine (10 μg) and 2-deoxy-D-glucose-treated group.

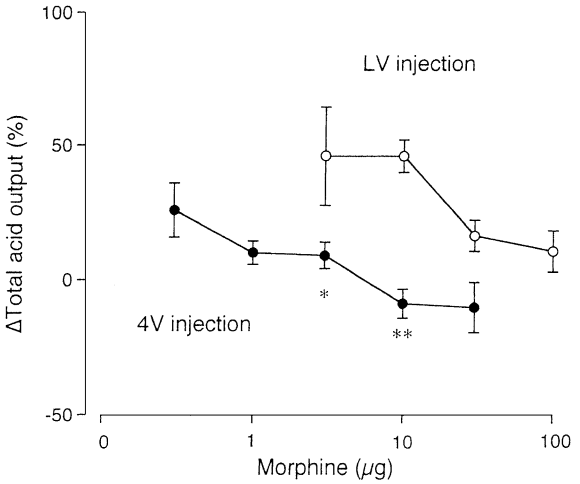

Fig. 2. Dose-dependent effect of morphine injected into fourth or lateral cerebroventricle on gastric acid secretion stimulated by 2-deoxy-D-glucose. Vehicle (5 μl) or morphine (0.3–100 μg/rat) was microinjected into the fourth (4V, ●) or lateral (LV, ○) cerebroventricle 10 min before the injection of 2-deoxy-D-glucose (200 mg/kg, i.v.). Values are standardized to the percentage of the total acid output for 120 min after 2-deoxy-D-glucose administration without morphine. Each value represents the mean ± S.E.M. for four to nine rats. $^{*}P < 0.05$, $^{**}P < 0.01$ statistically significant compared with lateral cerebroventricle-injected group.

Although the total acid output in the rat injected with morphine (10, 30 μg) appeared to be lower than the basal output, the findings were not significant because of a wide variation. Injection of DAMGO (1 μg, a peptidyl μ-opioid receptor agonist) into the fourth cerebroventricle also inhibited the 2-deoxy-D-glucose-stimulated secretion; the total acid outputs in the presence of 2-deoxy-D-glucose for 120 min in the control and the DAMGO-treated rats were 289.1 ± 35.0 μEq HCl ($n = 5$) and 107.9 ± 46.7 μEq HCl ($n = 4$, $P < 0.05$ compared with the control), respectively. Injection of naloxone (100 μg), a non-selective antagonist for opioid receptors, into the fourth cerebroventricle at 10 min before morphine injection significantly reversed the inhibitory effect of morphine (10 μg) (Fig. 1B). Injection of norbinaltorphimine (10 μg), a selective κ-opioid receptor antagonist (Birch et al., 1987), did not reverse the inhibitory effect of morphine. Intravenous injection of morphine (10 mg/rat) did not yield the inhibitory effect (data not shown).

For further investigation of the effective site in the CNS, morphine was injected into lateral cerebroventricle, a higher level of the brain than the fourth cerebroventricle. Compared with that of the fourth cerebroventricle injection, the inhibitory effect of morphine on lateral cerebroventricle injection was less effective at 3 and 10 μg (Fig. 2). The inhibitory effect of morphine injected into the lateral cerebroventricle was also restored by naloxone injected into the lateral cerebroventricle. Injection of morphine (3 and 10 μg) into the fourth or the lateral cerebroventricle itself had neither stimulatory or inhibitory effect on basal gastric acid secretion (data not shown). These findings suggest that morphine has an inhibitory effect on gastric acid secretion, and that this effect was more potent on injection into the fourth cerebroventricle, a subordinate position which also includes the brainstem and the medulla oblongata.

3.2. Effect of central injection of U69593, a κ-opioid receptor agonist, on gastric acid secretion

The fourth cerebroventricle injection of U69593, a selective κ-opioid receptor agonist (Lahti et al., 1985), increased gastric acid secretion in a dose-dependent manner (Fig. 3). Gastric acid secretion began to increase about 5–15 min after the injection of U69593, and gradually increased until the peak level was reached at 40–50 min. The acid output then returned to the baseline at 120 min. Furthermore, injection of U69593 (1, 3 μg) into the lateral cerebroventricle produced significantly more gastric acid secretion than fourth cerebroventricle injection (Fig. 4). Injection of U69593 (1 μg) into the lateral cerebroventricle, but not into the fourth cerebroventricle, increased acid secretion. The time course of gastric acid secretion after lateral cerebroventricle injection of U69593 was similar to that after fourth cerebroventricle injection. Injection of U69593 (3 μg) into the lateral cerebroventricle did not enhance the 2-deoxy-D-glucose-stimulated gastric acid secretion. U69593 was less effective to inhibit the gastric acid secretion stimulated by 2-deoxy-D-glucose than was morphine; the total acid outputs for 120 min after 2-deoxy-D-glucose administration in the U69593 (30 μg, lateral cerebroventricle)-treated group and the control group were

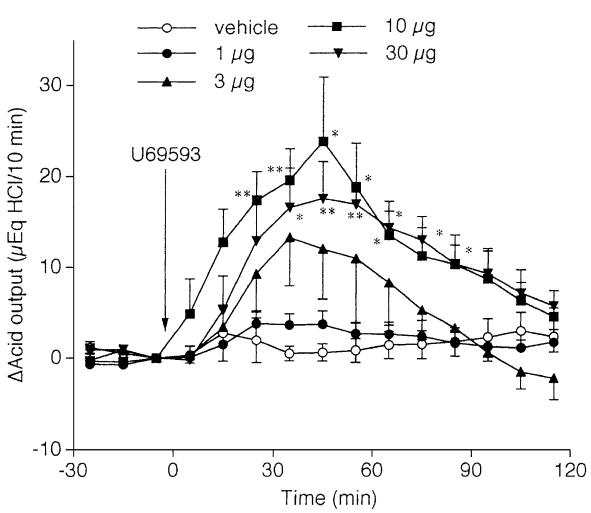

Fig. 3. Effect of U69593 injected into fourth cerebroventricle on gastric acid secretion. Vehicle (5 μl) or U69593 (1–30 μg/rat) was microinjected into fourth cerebroventricle. Each value represents the gastric acid output for 10 min. Each value is the mean ± S.E.M. for four to five rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ statistically significant compared with the control (vehicle) group.

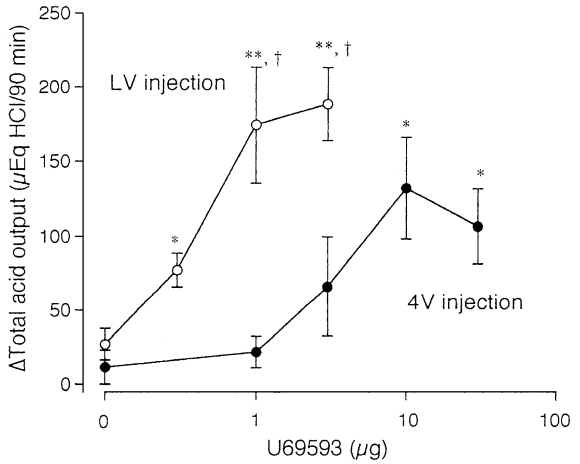

Fig. 4. Dose-dependent effect of U69593 injected into fourth or lateral cerebroventricle on gastric acid secretion. Vehicle (5 μl) or U69593 (0.3–30 μg/rat) was microinjected into the fourth (4V, ●) or the lateral (LV, ○) cerebroventricle. Values are expressed as the total gastric acid output for 90 min. Each value represents the mean ± S.E.M. for four to seven rats. $^{*}P < 0.05$, $^{**}P < 0.01$ statistically significant compared with the control group. †$P < 0.05$ compared with fourth cerebroventricle-injected group.

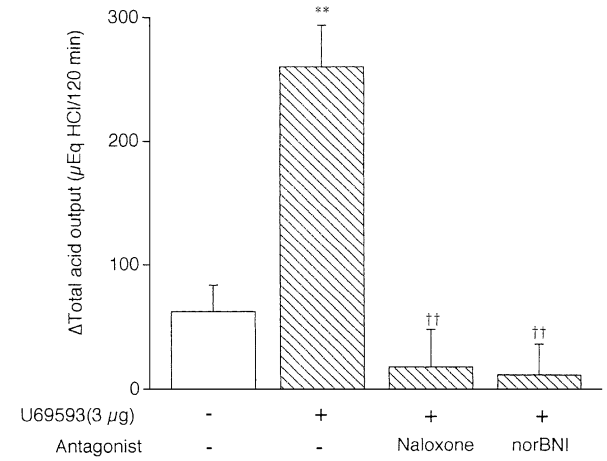

Fig. 5. Effect of naloxone and norbinaltorphimine on gastric acid secretion stimulated by U69593 injected into lateral cerebroventricle. Naloxone (30 μg/rat) and norbinaltorphimine (norBNI, 10 μg) were injected into the lateral cerebroventricle 10 min before injection of U69593 (3 μg). The total gastric acid output for 120 min is shown. Each column represents the mean ± S.E.M. for four to seven rats. $^{**}P < 0.01$ statistically significant compared with the control group. ††$P < 0.01$ compared with the U69593-treated group.

111.7 ± 45.7 μEq HCl and 171.7 ± 54.9 μEq HCl ($n = 3$), respectively (not significant, $P = 0.450$). Intravenous injection of U69593 (1 μg) did not stimulate secretion. To determine whether the stimulatory effect of U69593 was receptor-mediated, naloxone or norbinaltorphimine, a selective κ-opioid receptor antagonist (Birch et al., 1987), was administered into lateral cerebroventricle 10 min before injection of U69593. Both naloxone (30 μg) and norbinaltorphimine (10 μg) completely blocked the acid secretion induced by lateral cerebroventricle injection of U69593 (3 μg) (Fig. 5).

Next, we investigated the effect of U50488H, another κ-opioid receptor agonist (Von Voightlander et al., 1983). Lateral cerebroventricle injection of U50488H (10 μg) markedly stimulated gastric acid secretion similarly to U69593 (Fig. 6). Gastric acid secretion induced by U50488H showed a pattern similar to that with U69593; the secretion increased gradually, and reached the maximum level of 30 μEq HCl/10 min after 40–50 min. The secretion stimulated by U50488H was inhibited by 10-min preinjection of either naloxone (100 μg) (Fig. 6) or norbinaltorphimine (10 μg) (data not shown). Although the total acid secretion for 120 min was less than that after lateral cerebroventricle injection, U50488H (10 μg) injected into fourth cerebroventricle also significantly stimulated gastric acid secretion (Fig. 6). With the fourth cerebroventricle injection, gastric acid secretion increased slowly, and the maximum level of 20 μEq HCl/10 min was reached at 100 min after U50488H injection. Furthermore, the stimulatory effect continued over 3 h, and acid secretion did not return to its baseline level. Intravenous injection of U50488H (10 μg) did not stimulate the secretion. Bremazocine (1 and 3 μg), a selective agonist for κ_2-opioid receptor (Tiberi and Magnan, 1989), injected into the lateral cerebroventricle significantly stimulated gastric acid secretion (Fig. 7). The stimulatory effect of bremazocine (3 μg) was almost completely abolished by norbinaltorphimine (10 μg). These findings suggest that activation of κ-opioid receptor is positively involved in the central regulation of gastric acid secretion.

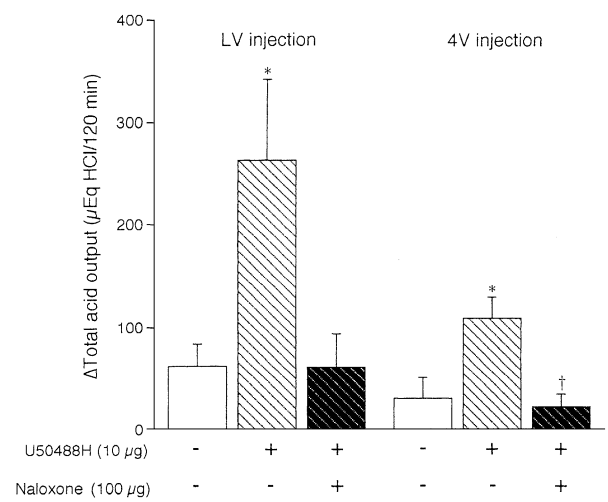

Fig. 6. Effect of U50488H injected into lateral or fourth cerebroventricle on gastric acid secretion. Saline (5 μl) and naloxone (30 μg/rat) were injected into the lateral (LV) or the fourth cerebroventricle (4V) at 10 min before injection of U50488H (10 μg). The total gastric acid output for 120 min is shown. Each column represents the mean ± S.E.M. for four to seven rats. $^{*}P < 0.05$ statistically significant compared with the control group. †$P < 0.05$ compared with the U50488H-treated group.

3.3. Role of the vagus nerve in the gastric acid secretion stimulated by the κ-opioid receptor agonists

Next, we investigated whether the vagus nerve was involved in the mechanism of the stimulatory effect of κ-opioid receptor stimulation by using two approaches, atropine administration and vagotomy. Atropine sulfate (1 mg/kg, i.v.) was administered 10 min before lateral cerebroventricle injection of U69593. The gastric acid secretion stimulated by U69593 (Fig. 8) and by U50488H (data not shown) 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 U69593 (Fig. 8) and U50488H (data not shown) was also completely inhibited in vagotomized rats. These findings suggest that the stimulatory effects of U50488H and U69593 on gastric acid secretion are mediated via the vagus cholinergic nerve.

3.4. Involvement of δ-opioid receptor in central regulation of gastric acid secretion

We also investigated the effect of DPDPE, a δ-opioid receptor agonist (Cotton et al., 1985), on basal and stimulated gastric acid secretion. Injection of DPDPE (10 μg) into either fourth or lateral cerebroventricle had no effect on basal or 2-deoxy-D-glucose-stimulated gastric acid secretion. The values were similar to the control values without DPDPE. For example, with lateral cerebroventricle injection, the total acid outputs for 120 min in the control and the DPDPE-treated rats were 33.9 ± 14.1 μEq HCl ($n = 5$) and 64.5 ± 27.3 μEq HCl ($n = 3$), respectively. The total outputs stimulated by 2-deoxy-D-glucose in the control and the DPDPE-treated rats were 318.5 ± 118.3 μEq HCl ($n = 3$) and 295.6 ± 161.4 μEq HCl ($n = 4$), respectively.

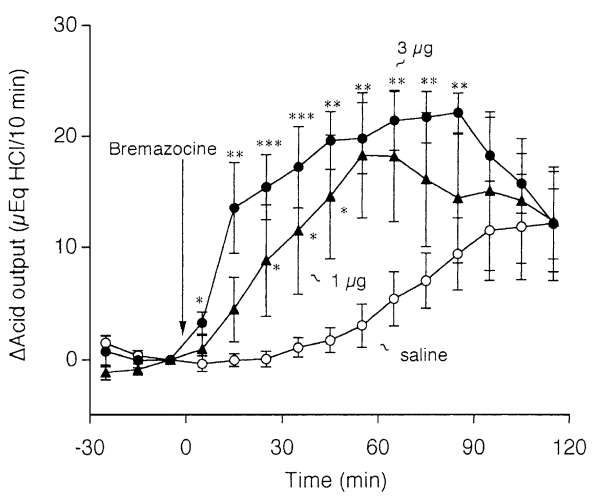

Fig. 7. Effect of bremazocine injected into lateral cerebroventricle on gastric acid secretion. Saline (5 μl, ○) or 1 (▲) and 3 (●) μg/rat of bremazocine was injected into lateral cerebroventricle at 0 min. Each value represents the gastric acid output for 10 min. Each value is the mean ± S.E.M. for four to seven rats. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ statistically significant compared with the control group.

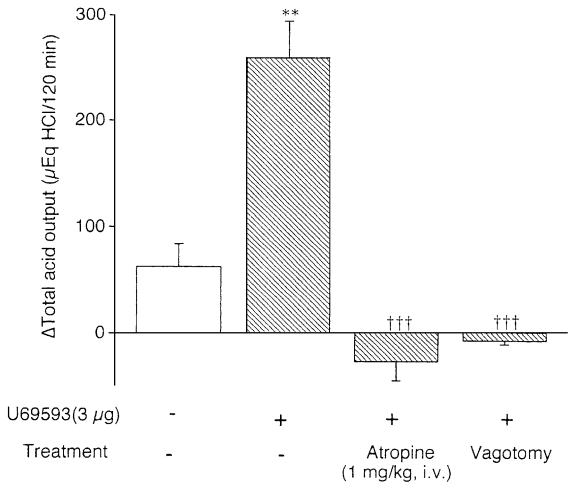

Fig. 8. Effect of atropine and vagotomy on gastric acid secretion stimulated by U69593 injected into lateral cerebroventricle. Atropine (1 mg/kg, i.v.) was injected 10 min before injection of U69593 (3 μg/rat). Bilateral vagotomy at cervical level or sham operation was performed 60 min before the experiment. The total gastric acid output for 120 min is shown. Each column represents the mean ± S.E.M. for four to five rats. $^{**}P < 0.01$ statistically significant compared with the control group. †††$P < 0.001$ compared with the U69593-treated group.

4. Discussion

4.1. Inhibition of gastric acid secretion by injection of morphine into the fourth cerebroventricle

Previously, we reported that the i.v. infusion of morphine (0.1 mg/kg per h) inhibited the gastric acid secretion stimulated by 2-deoxy-D-glucose in the perfused stomach of urethane-anesthetized rats (Watanabe et al., 1987). Since morphine did not inhibit the gastric acid secretion evoked peripherally by electrical stimulation of the vagus nerve, the inhibitory effect of morphine appeared to be mediated by the CNS (Watanabe et al., 1987). We now investigated the effect of morphine injected into fourth and lateral cerebroventricle on gastric acid secretion, to help clarify the effect of morphine on the CNS. The fourth cerebroventricle injection of morphine, which mainly acted on μ-opioid receptors, inhibited gastric acid secretion stimulated by 2-deoxy-D-glucose in a dose-dependent manner (Fig. 1). The inhibitory effect of morphine was reversed by preinjection of naloxone into the fourth cerebroventricle (Fig. 1B). In addition, DAMGO, a μ-opioid receptor agonist, also inhibited the secretion. With the i.c.v. injection, morphine inhibited gastric acid secretion in rats (Roze et al., 1980; Fox and Burks, 1988). The present and previous findings suggest that the inhibitory effect of morphine on gastric acid secretion was mediated by the μ-opioid receptors in the CNS.

The expression of mRNA and protein of μ-opioid receptors is much greater in the thalamus in the forebrain near the lateral cerebroventricle and the nucleus of the solitary tract in the brainstem than in other areas of the brain (Mansoure et al., 1995). Although the drugs given by fourth cerebroventricle injection spread only around the brainstem, the drugs given by lateral cerebroventricle injection spread widely around the forebrain and the caudal regions such as fourth cerebroventricle. In the present study, the lateral cerebroventricle injection of morphine was less effective than the fourth cerebroventricle injection (Fig. 2). The nucleus of the solitary tract is considered to be one of the nuclei involved in the regulation of gastric acid secretion (Osumi et al., 1981). Thus, the nucleus of the solitary tract appeared to be a candidate target of morphine.

4.2. Stimulation of gastric acid secretion by injection of κ-opioid receptor agonists into lateral cerebroventricle

In the present study, we showed that the central injection of U69593 and U50488H, κ-opioid receptor agonists, significantly stimulated gastric acid secretion, in contrast to morphine (Figs. 3 and 6). The lateral cerebroventricle injection of bremazocine, a κ_2-opioid receptor agonist, also stimulated gastric acid secretion (Fig. 7), and the stimulatory effects of U69593 and U50488H were antagonized by naloxone and by a selective κ-opioid receptor antagonist, norbinaltorphimine, under the present experimental conditions (Fig. 5). Since the effects of U69593 and U50488H were abolished in atropine-treated rats and in vagotomized rats, the effects appeared to be mediated by the vagus cholinergic nerve. Although Fox and Burks (1988) observed that i.v. injection of U50488H slightly stimulated gastric acid secretion in pylorus-ligated rats, they suggested that the effect of U50488H was not mediated by the κ-opioid receptor, because other κ-opioid receptor agonists such as dynorphin A-(1–17) did not stimulate gastric acid secretion. Our findings show the involvement of κ-opioid receptors in the CNS in positive regulation of gastric acid secretion.

As shown in Fig. 7, the basal gastric acid secretion appeared to increase slowly after lateral cerebroventricle injection of saline. The injection of norbinaltorphimine (10 μg) into the lateral cerebroventricle showed a slight inhibition of basal gastric acid secretion (1.43 ± 18.33 μEq HCl, $n = 6$, $P = 0.392$ compared with the control in Fig. 6). Further studies of the possible involvement of endogenous κ-opioid receptor ligand on gastric acid secretion are currently in progress in our laboratory.

It was reported that the i.c.v. injection of dynorphin, a peptidyl κ-opioid receptor agonist, tended to stimulate gastric acid secretion, but significantly inhibited the secretion stimulated by thyrotropin-releasing hormone (Morley et al., 1981). In the present study, however, U69593 did not inhibit 2-deoxy-D-glucose-stimulated gastric acid secretion. Thus, the dual, stimulatory and inhibitory, effects on gastric acid secretion may be induced by κ-opioid receptor stimulation in the CNS. As described below, different regions of the CNS may be involved in the two effects induced by κ-opioid receptor agonists. The effects of κ-opioid receptor agonists after lateral cerebroventricle injection were more potent than those after fourth cerebroventricle injection. The distribution of κ-opioid receptor mRNA in the hypothalamus is very high (Mansoure et al., 1995). In the ventromedial hypothalamus, the lateral hypothalamus area and the paraventricular nucleus, which are important nuclei in the hypothalamus for the regulation of gastric acid release (Shiraishi and Simpson, 1987), the mRNA and the proteins of the κ-opioid receptor expressed at a high density (Mansoure et al., 1995). Further investigations are necessary to determine the endogenous κ-opioid receptor agonists and the nuclei, which inhibit gastric acid secretion through κ-opioid receptors in the hypothalamus.

4.3. Physiological roles of μ-, δ- and κ-opioid receptor on gastric acid secretion

We also investigated the effect of DPDPE, a selective δ-opioid receptor agonist, on basal and 2-deoxy-D-glucose-stimulated gastric acid secretion. DPDPE did not regulate the secretions, whether injected into fourth cerebroventricle or lateral cerebroventricle. Fox and Burks (1988) reported that neither i.v. nor i.c.v. injection of DPDPE showed any effect on gastric acid secretion in pylorus-ligated rats, and Improta and Broccardo (1994) reported that i.c.v. injection of a selective δ_2-opioid receptor agonist had no effect on the secretion in normal rats. From the present and previous findings, it appears that the δ-opioid receptor system is not involved in the central regulation of gastric acid secretion.

The nuclei, which are possible sites that regulate gastric acid secretion as described above, were shown to play important roles in the regulation of food intake (Gosnell et al., 1986). Centrally injected orexin and neuropeptide Y stimulated food intake and gastric acid secretion via the CNS (Geoghegan et al., 1993; Takahashi et al., 1999; Inui, 1999). In the present study, we demonstrated that gastric acid secretion was stimulated by activation of the κ-opioid receptors in the CNS. The effect of κ-opioid receptor activation on food intake should be determined in the future.

4.4. Summary

In conclusion, central injection of κ-opioid receptor agonists stimulated gastric acid secretion, via the receptors and through vagus nerve mechanisms, in the perfused stomach of urethane-anesthetized rats. Injection of morphine, a μ-opioid receptor agonist, inhibited the secretion, and DPDPE, a δ-opioid receptor agonist, had no effect on the secretion. The stimulatory effect of κ-opioid receptor

agonists on gastric acid secretion was more potent on injection into the lateral cerebroventricle than into the fourth cerebroventricle, but the inhibitory effect of the μ-opioid receptor agonist was more potent after injection into the fourth cerebroventricle than after injection into the lateral cerebroventricle.

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