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Involvement of glutamate and γ-amino-butyric acid receptor systems on gastric acid secretion induced by activation of κ-opioid receptors in the central nervous system in rats

[1]Sachie Minowa, [1]Satomi Ishihara, [1]Shizuko Tsuchiya, [1]Syunji Horie, [1]Kazuo Watanabe & *,[1]Toshihiko Murayama

[1]Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 263-8522, Japan

1 Various neurotransmitters in the brain regulate gastric acid secretion. Previously, we reported that the central injection of κ-opioid receptor agonists stimulated this secretion in rats. Although the existence of κ_1–κ_3-opioid receptor subtypes has been proposed, the character is not defined. We investigated the interactions between κ-opioid receptor subtypes and glutamate, γ-amino-butyric acid (GABA) or 5-hydroxy tryptamine (5-HT) receptors in the rat brain.

2 Gastric acid secretion induced by the injection of U69593 (8.41 nmol, a putative κ_1-opioid receptor agonist) into the lateral cerebroventricle was completely inhibited by the central injection of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10.9 nmol, an antagonist for non-*N*-methyl-D-aspartate (non-NMDA) receptors) and by bicuculline infusion ($222\,\mu g\,kg^{-1}$ per 10 min, i.v., $GABA_A$ receptor antagonist). The secretion induced by bremazocine (8.52 nmol, a putative κ_2-opioid receptor agonist) was inhibited by bicuculline infusion, but not by CNQX. The secretion induced by naloxone benzoylhydrazone (224 nmol, a putative κ_3-opioid receptor agonist) was slightly and partially inhibited by CNQX and bicuculline.

3 Treatment with CNQX and bicuculline inhibited gastric acid secretion induced by the injection of dynorphin A-(1-17) into the lateral, but not the fourth, cerebroventricle. Antagonists for NMDA, $GABA_B$ and $5\text{-}HT_{2/1C}$ receptors did not inhibit the secretions by κ-opioid receptor agonists.

4 In rat brain regions close to the lateral cerebroventricle, κ-opioid receptor systems ($\kappa_1 > \kappa_3 \gg \kappa_2$) are regulated by the non-NMDA type of glutamate receptor system, and κ_1- and κ_2-opioid receptor systems are regulated by the $GABA_A$ receptor system. The present findings show pharmacological evidence for κ-opioid receptor subtypes that regulate gastric acid secretion in the rat brain.

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Keywords: Gastric acid secretion; opioid receptor subtype; glutamate, γ-amino-butyric acid; central injection

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione sodium salt; CNS, central nervous system; CPP, (±)-3-[2-carboxypiperazin-4-yl]-1-propylphosphonic acid; GABA, γ-amino-butyric acid; NBH, naloxone benzoylhydrazone; NMDA, *N*-methyl-D-aspartate; U69593, (5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide; 5-HT, 5-hydroxy tryptamine

Introduction

Recent advances have shown that gastric functions including acid secretion are regulated not only by the peripheral but also by the central nervous system (CNS) (Fox & Burks, 1988; Grijalva & Novin, 1990; Taché *et al.*, 1991; Geoghegan & Pappas, 1997). Many studies concerning the central effects of various neurotransmitters and neuropeptides on gastric acid secretion have revealed mechanisms by which the brain regulates gastric acid secretion (Yang *et al.*, 1993; Yoneda & Taché, 1995; Geoghegan & Pappas, 1997). Glutamate is a primary excitatory neurotransmitter in the CNS, and subtypes of ionotropic glutamate receptors have been classified as *N*-methyl-D-aspartic acid (NMDA), kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. It has been reported that the central injection of NMDA and kainate potently stimulated gastric acid secretion in rats (Kaneko & Taché, 1995; Yang *et al.*, 2000). In addition, we reported that the injection of kainate, but not AMPA, into the lateral cerebroventricle stimulated secretion in the perfused stomach of urethane-anesthetized rats (Tsuchiya *et al.*, 2001). It was reported that the injection of muscimol, an agonist for the A-type receptor of γ-amino-butyric acid (GABA), into the lateral cerebroventricle (i.c.v. administration) stimulated gastric acid secretion in rats (Del Tacca *et al.*, 1990; Namiki *et al.*, 1993; Lin, 1995). Neuroactive steroids and anesthetics such as pentobarbital have been confirmed to bind potently and selectively to ionotropic $GABA_A$ receptors in the CNS (Lambert *et al.*, 1995). Previously, we reported that i.v. injection of anesthetics (Lin *et al.*, 1988) and the injection of neuroactive progesterone metabolites into the lateral

*Author for correspondence; E-mail: murayama@p.chiba-u.ac.jp

cerebroventricle (Watanabe *et al.*, 2000) stimulated gastric acid secretion centrally in rats. The importance of the lateral hypothalamus close to the lateral cerebroventricle in the control of gastric function has been established (Taché, 1987; Stanley *et al.*, 1997). The NMDA and kainate receptors (Van Den Pol *et al.*, 1994; Vignes and Collingridge, 1997; Eyigor *et al.*, 2001) and $GABA_A$ receptors (Pirker *et al.*, 2000) are distributed throughout the rat brain, including the hypothalamus, although a heterogeneous distribution of the receptor subunits was observed. These findings showed the involvement of NMDA, kainate and $GABA_A$ receptors in the CNS on gastric acid secretion.

Gastric acid secretion is also regulated by the opioid system in the CNS. Fox & Burks (1988) reported that the i.c.v. injection of μ-opioid receptor agonists such as morphine inhibited gastric acid secretion, and we reported that the injection of morphine into the fourth cerebroventricle inhibited secretion in rats (Ishihara *et al.*, 2001a). Although Fox & Burks (1988) reported that κ-opioid receptor agonists did not produce a significant change in gastric acid secretion after i.c.v. injection in rats, we reported that the injection of dynorphin A-(1-17) (an endogenous and nonselective agonist for κ-opioid receptors) into the lateral cerebroventricle stimulated gastric acid secretion in rats (Ishihara *et al.*, 2001b). The mRNA and the proteins of the κ-opioid receptor are expressed at a high density in the rat hypothalamus (Mansoure *et al.*, 1995). The existence of κ-opioid receptor subtypes has been proposed; arylacetamides, such as (5α,7α,8β)-(+)-*N*-methyl-*N*-(7-[1-pyrrolidinyl]-1-oxaspiro[4.5]dec-8-yl)benzeneacetamide (U69593), bind to a subset of sites (κ_1), and the κ_2 site has moderate affinity for bremazocine, and naloxone benzoylhydrazone (NBH) labels a population of distinct sites (κ_3) under κ-opioid receptor-selective conditions (Satoh & Minami, 1995; Dhawan *et al.*, 1996; Law & Loh, 1999). Although it was suggested that the cloned κ-opioid receptor corresponds to the κ_1 subtype and that the κ_2 subtype resulted from a heterodimer of κ_1- and δ-(Jordan & Devi, 1999) or μ-opioid receptors (Simonin *et al.*, 2001), cloning of each subtype and functional characterization are necessary to support the proposed subtypes. We reported that the injections of U69593 (a putative agonist for the κ_1-opioid receptor), bremazocine (for κ_2) and NBH (for κ_3) into the lateral cerebroventricle stimulated gastric acid secretion in rats (Ishihara *et al.*, 2001a, b). However, it has not been established whether these three proposed agonists for κ_1–κ_3-opioid receptors activate the respective subtypes specifically in the CNS. In addition, it has not been determined whether the κ-opioid receptor system interacts with other receptor systems such as glutamate and GABA receptors in the CNS and thus stimulates gastric acid secretion in rats.

In this study, we investigated the involvement of glutamate, GABA and the 5-hydroxy tryptamine (5-HT) receptor system on gastric acid secretion stimulated by the respective agonists for κ_1–κ_3-opioid receptors in the CNS in the perfused stomach of urethane-anesthetized rats. It is proposed that gastric acid secretion induced by the injection of κ-opioid receptor agonists into the lateral cerebroventricle is mediated by the non-NMDA type of glutamate receptor and $GABA_A$ receptor systems, and that the contributions of both receptor systems are different between the subtypes of the κ-opioid receptors in the CNS in rats. To our knowlege, this is the first report that pharmacologically shows the existence of subtypes of κ-opioid receptors that regulate gastric acid secretion in the rat brain.

Methods

Animals

Male Wistar rats (Takasugi Exp. Animals, Kasukabe, 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 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.

Drugs

Dynorphin A-(1-17) was purchased from Peptide Institute Co. (Osaka, Japan). U69593, (±)-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), kainate and (±)-3-[2-carboxypiperazin-4-yl]-1-propylphosphonic acid (CPP) were obtained from RBI (Natic, MA, USA). NBH, 6-cyano-7-nitroquinoxaline-2,3-dione sodium salt (CNQX), bicuculline, 3-amino-2-(4-chlorophenyl)propylphosphonic acid (phaclofen) and 3-[2-(4-[4-fluorobenzoyl]-1-piperidinyl)ethyl] 2,4 [1H. 3H]-quinazolinedione (ketanserin) were obtained from Sigma Chemical Co. (St Louis, MO, USA).

The doses of respective antagonists were as previously reported (Lin *et al.*, 1989; Watanabe *et al.*, 2000; Tsuchiya *et al.*, 2001). U69593 and phaclofen were dissolved in a minimum of 0.1 N HCl and saline, and the final pH was about 4.5. NBH was dissolved in a minimum of 3% acetic acid and saline (pH 4.5). Dynorphin A-(1-17), bremazocine, CNQX, CPP and ketanserin were dissolved in saline. The agonists for κ-opioid receptors, CNQX, CPP, kainate, phaclofen and ketanserin, were administered in a volume of 5 μl over 30 s using a microliter syringe through a guide cannula positioned in the brain. The vehicles and saline had no effect on gastric acid secretion by themselves. Bicuculline was dissolved in a minimum of 1 N HCl, and diluted by saline, adjusted with 1 N NaOH, and the final pH was about 4.5. Bicuculline (222 μg kg^{-1} per 10 min) was infused through an i.v. cannula inserted into the femoral vein.

Cannulation for central or i.v. injection

Cannulation for the central injection was performed as previously reported (Ishihara *et al.*, 2001a, b). Briefly, the rats were anesthetized with urethane (1.35 g kg^{-1}, i.p.). Then 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 the microinjection of drugs was implanted into the lateral cerebroventricle with the following coordinates taken from the atlas of Paxinos & Watson (1997): 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 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. In some animals, the femoral vein was cannulated for intravenous infusion.

Measurement of gastric acid secretion

Each experiment was started at least 1 h after the implantation of the cannulas. This procedure is common in research concerning short-term neuronal regulation of gastric acid secretion (Watanabe *et al.*, 2000; García-Zaragozá *et al.*, 2000). Gastric acid secretion was determined by the gastric perfusion methods as previously reported (Watanabe *et al.*, 2000; Ishihara *et al.*, 2000a, b). 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^{-1}. 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. Antagonists for each receptor except bicuculline were administrated 10 min before the injection of κ-opioid receptor agonists. Bicuculline was infused i.v. from 30 min before injection of agonists for κ-opioid receptors and the infusion continued for 90 or 120 min. After 30 min of pre-perfusion, 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). The acid output was expressed in terms of μEq HCl per 10 min. In some experiments, the total acid output for 90 min was measured.

Statistical analysis

The values are expressed as means ± s.e.m. for three to eight 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.

Results

Effects of antagonists of glutamate receptors on gastric acid secretion stimulated by the injection of κ-opioid receptor agonists into the lateral cerebroventricle

Previously, we reported that the injection of various agonists for κ-opioid receptors into the lateral cerebroventricle stimulated gastric acid secretion in a dose-dependent manner in rats (Ishihara *et al.*, 2001a, b). In the present study, we used the indicated doses of respective agonists for κ-opioid receptors, which were almost maximal doses, and stimulated gastric acid secretion to a similar degree. First, we investigated the effect of CNQX (an antagonist for non-NMDA receptors) on gastric acid secretion stimulated by κ-opioid receptor agonists. The injection of CNQX (10.9 nmol (3 μg) per rat) into the lateral cerebroventricle inhibited gastric acid secretion stimulated by the central injection of kainate (0.47 nmol (0.1 μg) per rat) (Tsuchiya *et al.*, 2001). The central injection of CNQX (10.9 nmol) alone slightly stimulated gastric acid secretion during the period of 0–40 min after administration (Figure 1), as reported previously (Tsuchiya *et al.*, 2001). The injection of CNQX partially inhibited gastric acid secretions stimulated by dynorphin A-(1-17) (0.47 nmol) and NBH (224 nmol) (Figure 1a and d). The total acid outputs for 90 min stimulated by dynorphin A-(1-17) and NBH in the CNQX-treated rats were about half those in the control rats without CNQX (Table 1). The injection of CNQX almost completely inhibited the secretion stimulated by U69593 (8.41 nmol, Figure 1b). However, the injection of CNQX did not show an inhibitory effect on the secretion stimulated by bremazocine (8.52 nmol, Figure 1c).

The injection of CPP (39.7 nmol (10 μg) per rat, an antagonist for the NMDA receptor) into the lateral cerebroventricle inhibited gastric acid secretion stimulated by the central injection of NMDA, but not by kainate (Tsuchiya *et al.*, 2001). The central injection of CPP (39.7 nmol) did not inhibit the secretions stimulated by dynorphin A-(1-17), U69593 and NBH (Figure 2). The injection of CPP slightly enhanced the secretion stimulated by bremazocine (Figure 2c).

Effects of antagonists of GABA receptors on gastric acid secretion stimulated by the injection of κ-opioid receptor agonists into the lateral cerebroventricle

It was reported that bicuculline is a selective antagonist for $GABA_A$ receptors and that peripheral injection of bicuculline showed central effects (Mitsushima *et al.*, 1999; Lapchak *et al.*, 2000). In the present study, we investigated the effect of i.v. infusion of bicuculline (222 μg kg^{-1} 10 min^{-1}) from 30 min before the injection of agonists for κ-opioid receptors (Figure 3). The bicuculline infusion partially inhibited gastric acid secretion stimulated by dynorphin A-(1-17) (panel a). Interestingly, the inhibition of the secretion in the period of 0–40 min after the injection of dynorphin A-(1-17) by bicuculline infusion was limited and not significant; the total acid outputs stimulated by dynorphin A-(1-17) for 0–40 min were 52.3 ± 9.5 and 24.9 ± 10.3 μEq HCl per 40 min ($P=0.082$, $n=4$–5) in the control and bicuculline-treated rats, respectively. Bicuculline infusion markedly decreased the secretion during the late phase (during the period of 40–90 min following administration). The total acid output for 90 min in the bicuculline-treated rats was significantly inhibited compared with that in the control rats without bicuculline (Table 2). Bicuculline infusion almost completely inhibited the secretions stimulated by U69593 and bremazocine (panels b and c). The bicuculline infusion tended to show an inhibitory effect on the secretion stimulated by NBH (panel d). Similar to the secretion stimulated by dynorphin A-(1-17), bicuculline infusion did not inhibit the secretion stimulated by NBH during the initial phase; the total gastric acid outputs stimulated by NBH during the period of 0–40 min were 41.3 ± 6.8 and 36.3 ± 17.0 μEq HCl 40 min^{-1} ($P=0.79$, $n=5$) in the control and the bicuculline-treated rats, respectively. Although bicuculline infusion inhibited the secretion during the late phase, the total acid output for 90 min stimulated by NBH in the bicuculline-treated rats was not significant compared with that in the control rats (Table 2).

Next, we investigated the effect of phaclofen, a selective antagonist for $GABA_B$ receptors (Del Tacca *et al.*, 1990;

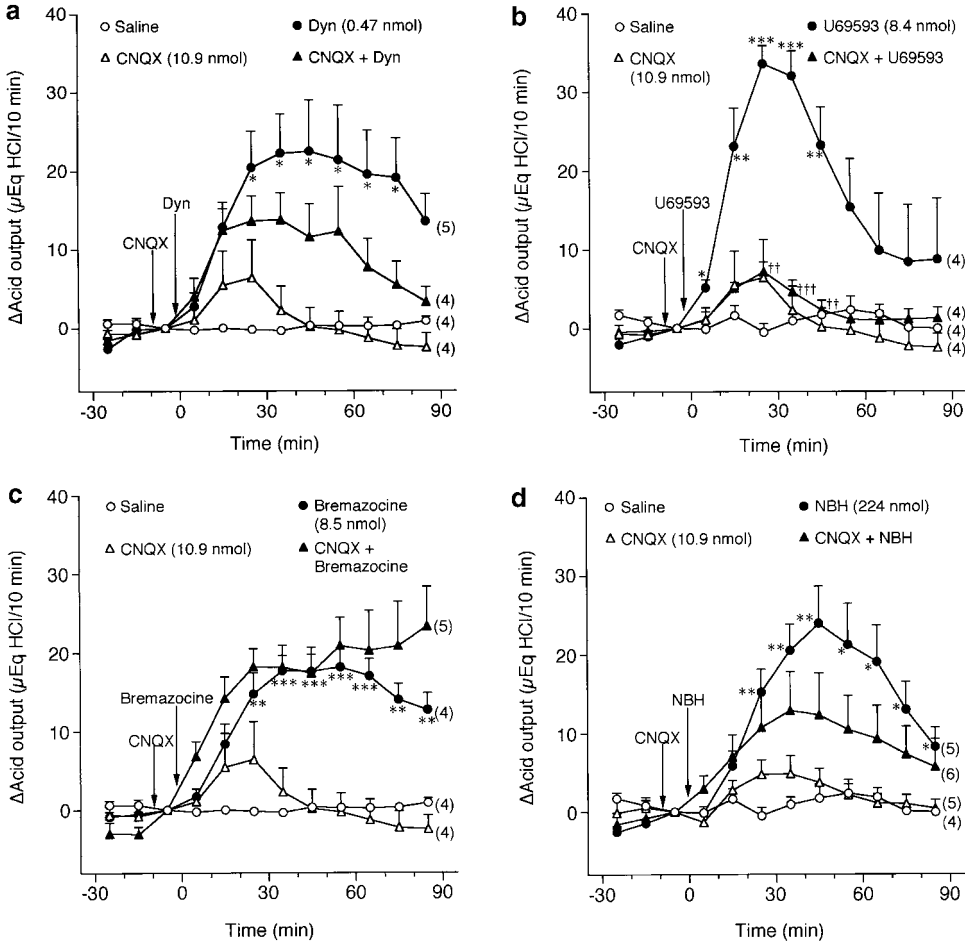

Figure 1 Effect of the central injection of CNQX on gastric acid secretion stimulated by injections of dynorphin A-(1-17) and selective agonists for κ_1–κ_3-opioid receptors into the lateral cerebroventricle. Vehicle (saline, 5 μl) or CNQX (10.9 nmol per rat, 5 μl) was injected into the lateral cerebroventricle at 10 min before the central injection of dynorphin A-(1-17) (panel a, Dyn, 0.47 nmol), U69593 (panel b, 8.41 nmol), bremazocine (panel c, 8.52 nmol) or NBH (panel d, 224 nmol). Each value represents the amount of gastric acid output for 10 min. Each value is the mean ± s.e.m. for four to six rats. *$P<0.05$, **$P<0.01$, ***$P<0.001$, statistically significant compared with the control (vehicle) group. $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, statistically significant compared with the respective agonist-treated group without CNQX. In Table 1, values are summarized as the total gastric acid output for 90 min.

Table 1 Effects of the central injection of CNQX and CPP on gastric acid secretions stimulated by the injections of agonists for κ-opioid receptors into the lateral cerebroventricle

	Total acid output (μEq HCl 90 min^{-1})		
Treatment	*Vehicle*	*CNQX*	*CPP*
Vehicle	−1.8 ± 2.6	9.0 ± 23.2	39.1 ± 17.6
Dynorphin A-(1-17)	145.5 ± 27.7*	84.0 ± 28.1	99.5 ± 26.9
U69593	159.3 ± 32.2*	24.7 ± 6.3**	175.3 ± 10.0
Bremazocine	122.0 ± 17.1*	160.0 ± 24.1	145.4 ± 53.7
NBH	126.7 ± 16.9*	78.4 ± 30.6	115.6 ± 17.5

Values are summarized from Figures 1 and 2. Each value is the total gastric acid output for 90 min and the mean ± s.e.m. for three to eight rats. *$P<0.01$, statistically significant compared with the control group. **$P<0.01$, statistically significant compared with the U69593-treated group without CNQX.

Table 2 Effects of antagonists for $GABA_A$ receptor and $GABA_B$ receptor on gastric acid secretions stimulated by the injections of agonists for κ-opioid receptors into the lateral cerebroventricle

	Total acid output (μEq HCl 90 min^{-1})		
Treatment	*Vehicle*	*Bicuculline*	*Phaclofen*
Vehicle	7.7 ± 5.6	1.4 ± 1.4	59.7 ± 53.0
Dynorphin A-(1-17)	145.5 ± 27.7	35.6 ± 21.3*	144.8 ± 30.4
U69593	159.3 ± 32.2	21.5 ± 14.5*	Not determined
Bremazocine	122.0 ± 17.1	−12.8 ± 23.0*	Not determined
NBH	126.7 ± 16.9	70.5 ± 28.9	177.1, 203.7

Values are summarized from Figures 3 and 4. Each value is the total gastric acid output for 90 min and the mean ± s.e.m. for three to six rats. *$P<0.01$, compared with the respective agonist-treated group without bicuculline. The values for NBH plus phaclofen were obtained from two rats ($n=2$).

Lambert *et al.*, 1995). As shown in Figure 4, the injection of phaclofen (120 nmol per rat) into the lateral cerebroventricle did not inhibit gastric acid secretion stimulated by dynorphin A-(1-17). The central injection of phaclofen also did not show an inhibitory effect on the secretion stimulated by NBH (Table 2).

Effect of intravenous infusion of bicuculline on gastric acid secretion stimulated by the injection of kainate into the lateral cerebroventricle

Next, we investigated whether the non-NMDA receptor system, which is activated by kainate, is located upstream from the $GABA_A$ receptor system. The injection of kainate (0.47 nmol) into the lateral cerebroventricle markedly stimulated gastric acid secretion (Figure 5), as reported previously (Tsuchiya *et al.*, 2001). The bicuculline infusion ($222\,\mu g\,kg^{-1}\,10\,min^{-1}$) significantly inhibited the secretion stimulated by kainate. It inhibited secretion both during the initial (0–40 min) and the late (40–90 min) phase.

Effect of ketanserin, an antagonist for $5HT_{2/1C}$ receptor, on gastric acid secretion stimulated by the injection of dynorphin A-(1-17) into the lateral cerebroventricle

It was reported that the 5-HT receptor system regulated gastric acid secretion in the CNS, and that among the 5-HT

Table 3 Effects of CNQX and bicuculline on gastric acid secretions stimulated by the injections of dynorphin A-(1-17) and U69593 into the fourth cerebroventricle

	Total acid output (μEq HCl 90 min^{-1})		
Treatment	*Vehicle*	*CNQX*	*Bicuculline*
Vehicle	4.6±5.4	55.6±9.4	1.4±1.3
Dynorphin A-(1-17)	153.5±41.6*	163.3±57.5	Not determined
U69593	132.0±33.8*	104.0±22.2	119.3±15.6

Vehicle (saline, 5 μl) or CNQX (10.9 nmol, 5 μl) were injected into the fourth cerebroventricle at 10 min before the injection of dynorphin A-(1-17) or U69593 into the fourth cerebroventricle. In some cases, saline or bicuculline ($222\,\mu g\,kg^{-1}\,10\,min^{-1}$, i.v.) were infused from 30 min before the injection of dynorphin A-(1-17) or U69593 into the fourth cerebroventricle, and for 60 min. The doses of dynorphin A-(1-17) and U69593 were 1.40 nmol and 28.1 nmol, respectively. Each value is the total acid output for 90 min and the mean±s.e.m. for three to four rats. *$P<0.01$, compared with the control group.

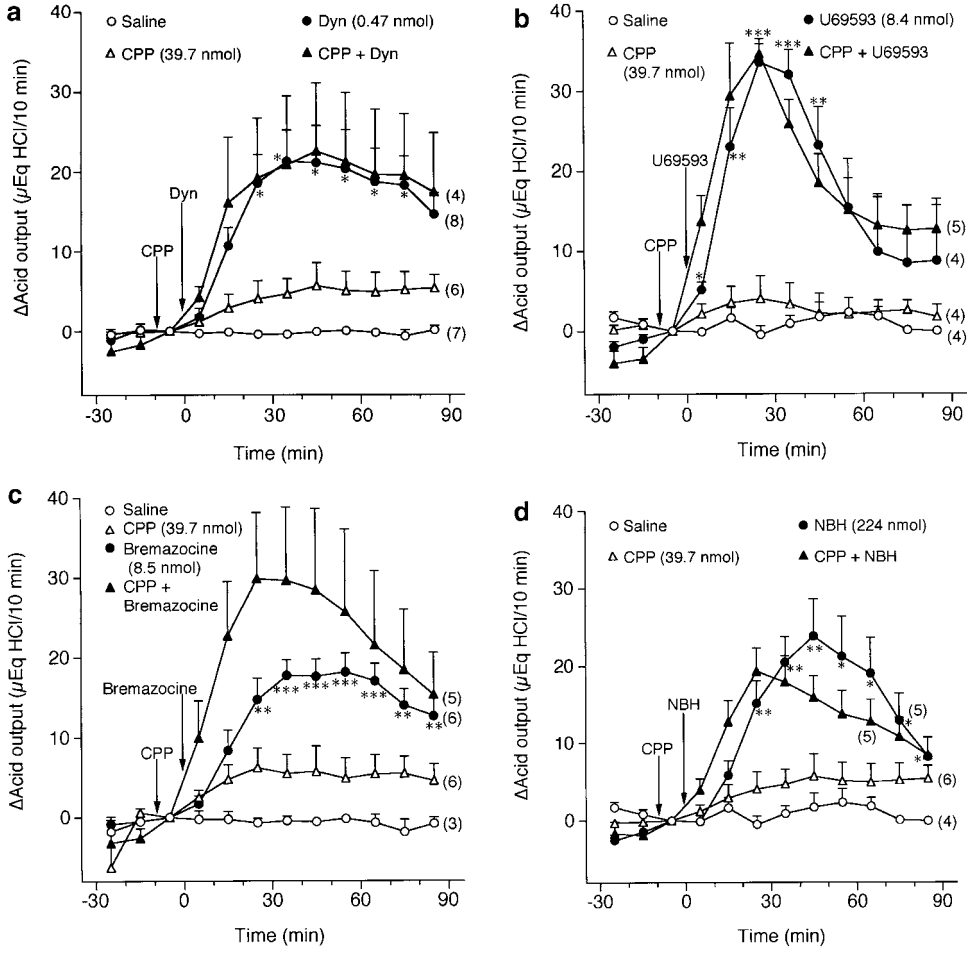

Figure 2 Effect of the central injection of CPP on gastric acid secretion stimulated by injections of dynorphin A-(1-17) and selective agonists for κ_1–κ_3-opioid receptors into the lateral cerebroventricle. Vehicle or CPP (39.7 nmol) was injected into the lateral cerebroventricle at 10 min before the central injection of dynorphin A-(1-17) (panel a, Dyn, 0.47 nmol), U69593 (panel b, 8.41 nmol), bremazocine (panel c, 8.52 nmol) or NBH (Panel d, 224 nmol). Each value represents the amount of gastric acid output for 10 min. Each value is the mean±s.e.m. for three to eight rats. *$P<0.05$, **$P<0.01$, ***$P<0.001$, compared with the control group. In Table 1, values are summarized as the total gastric acid output for 90 min.

Table 4 Subtype-dependent interactions between κ-opioid, glutamate and GABA receptor systems in the brain regions, which regulate gastric acid secretion close to the lateral cerebroventricle in rats

	Glutamate receptor dependency		*GABA receptor dependency*		
Subtypes	*NMDA*	*Non-NMDA (kainate)*	*$GABA_A$*		*$GABA_B$*
			(0–40 min)	*(40–90 min)*	
Dynorphin A-(1-17)	Not	Half	Half	Largely	Not
U69593 (κ_1)	Not	Largely	Largely	Largely	?
Bremazocine (κ_2)	Not	Not	Largely	Largely	?
NBH (κ_3)	Not	Half	Not	Half	Not

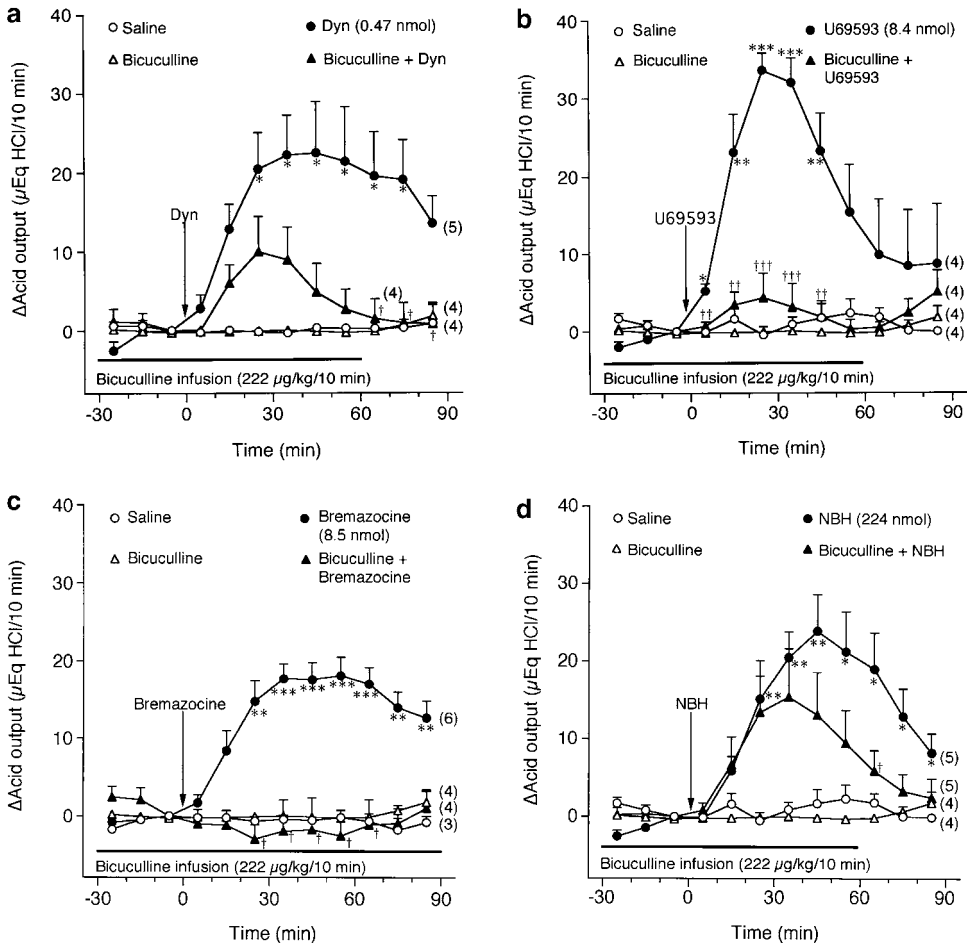

Figure 3 Effect of the intravenous infusion of bicuculline on gastric acid secretion stimulated by injections of dynorphin A-(1-17) and selective agonists for κ_1–κ_3-opioid receptors into the lateral cerebroventricle. Vehicle (saline) and bicuculline (222 μg (605 nmol) kg^{-1} 10 min^{-1}, i.v.) were infused from 30 min before the injection of dynorphin A-(1-17) (panel a, Dyn, 0.47 nmol), U69593 (panel b, 8.41 nmol), bremazocine (panel c, 8.52 nmol) or NBH (panel d, 224 nmol) into the lateral cerebroventricle. Each value represents the amount of gastric acid output for 10 min. Each value is the mean ± s.e.m. for three to six rats. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared with the control group. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, compared with the respective agonist-treated group without bicuculline. In Table 2, values are summarized as the total gastric acid output for 90 min.

receptor subtypes the 5-HT_2 receptor is more important (Yoneda and Taché, 1995; Chi *et al.*, 1996; Yang *et al.*, 2000). However, the injection of ketanserin (5.5 nmol per rat, a selective antagonist for 5-HT_2 and 5-HT_{1C} receptors) into the lateral cerebroventricle affected neither the basal gastric acid secretion (data not shown) nor the secretion stimulated by dynorphin A-(1-17) (0.47 nmol); the total acid outputs for 90 min were 154.1 ± 36.2 and 137.4 ± 30.5 μEq HCl ($n = 3$–5) in the control and ketanserin-treated rats, respectively.

Effects of CNQX and bicuculline on gastric acid secretion stimulated by the injection of U69593 into the fourth cerebroventricle

Previously, we reported that the injection of a higher dose (3–10-fold) of agonists for κ-opioid receptors into the fourth cerebroventricle, compared with the injection into the lateral cerebroventricle, stimulated gastric acid secretion in rats (Ishihara *et al.*, 2001a, b). Injection of a higher dose of

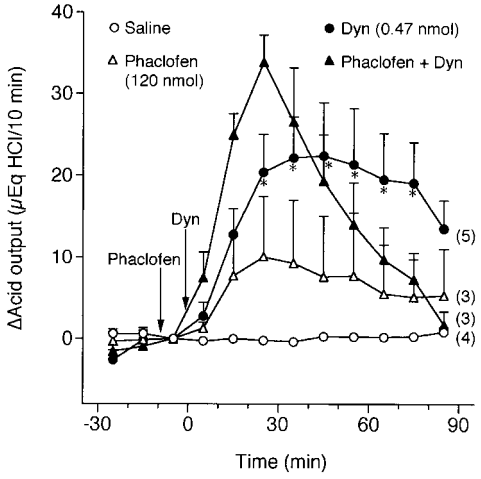

Figure 4 Effect of the central injection of phaclofen on gastric acid secretion stimulated by the injection of dynorphin A-(1-17) into the lateral cerebroventricle. Vehicle or phaclofen (120 nmol) was injected into the lateral cerebroventricle at 10 min before the central injection of dynorphin A-(1-17) (Dyn, 0.47 nmol). Each value represents the amount of gastric acid output for 10 min. Each value is the mean ± s.e.m. for three to five rats. $^{*}P<0.05$, compared with the control group. In Table 2, values are summarized as the total gastric acid output for 90 min.

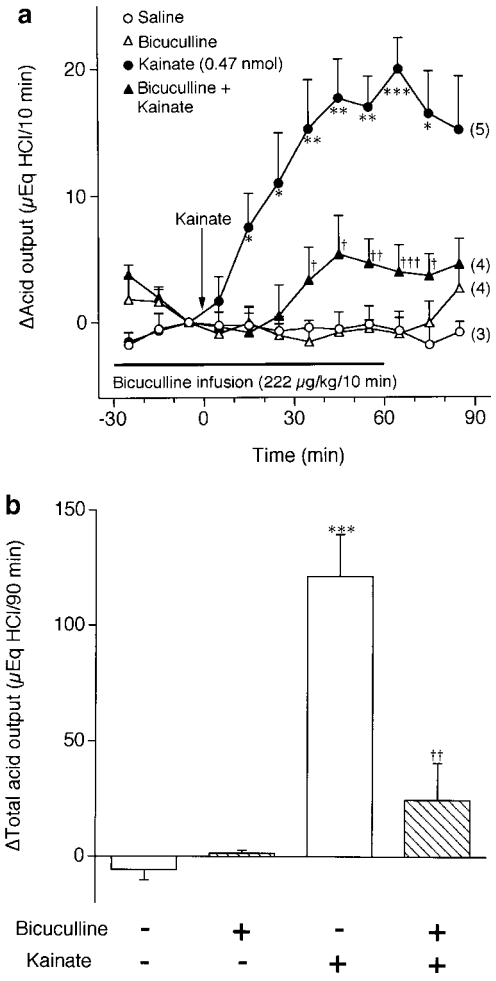

Figure 5 Effect of the intravenous infusion of bicuculline on gastric acid secretion stimulated by the injection of kainate into the lateral cerebroventricle. Vehicle (saline) and bicuculline ($222\,\mu g\,kg^{-1}\,10\,min^{-1}$, i.v.) were infused from 30 min before the injection of kainate (0.47 nmol) into the lateral cerebroventricle. (a) Each value represents the amount of gastric acid output for 90 min. (b) Each value is the total gastric acid output for 90 min. Each value is the mean ± s.e.m. for three to five rats. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, compared with the control group. $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, compared with the kainate-treated group without bicuculline.

U69593 (28.1 nmol) into the fourth cerebroventricle stimulated gastric acid secretion (Table 3) to the same degree obtained by the injection of a low dose (8.41 nmol) into the lateral cerebroventricle (Table 1). The time course of gastric acid secretion by the injection of U69593 into the fourth cerebroventricle (data not shown, but see Ishihara *et al.*, 2001a) was similar to that by injection into the lateral cerebroventricle. In both cases, gastric acid secretion began to increase about 5–15 min after the injection, and gradually increased until the peak level was reached at 30–40 min. The injection of CNQX (10.9 nmol) into the fourth cerebroventricle stimulated secretion immediately after the injection of CNQX. Although the reasons were unknown, the effect of CNQX injected into the fourth cerebroventricle was marked compared with that into the lateral cerebroventricle. The injection of CNQX did not appear to inhibit the secretion stimulated by U69593 (Table 3). In addition, total gastric acid secretion stimulated by the injection of dynorphin A-(1-17) (1.40 nmol) into the fourth cerebroventricle was not inhibited by CNQX. An intravenous infusion of bicuculline ($222\,\mu g\,kg^{-1}\,10\,min^{-1}$) did not inhibit gastric acid secretion stimulated by the injection of U69593 into the fourth cerebroventricle. Gastric acid secretion in the 90–150 min after the injection of U69593 was not inhibited by CNQX and bicuculline treatment (data not shown).

Discussion

Involvement of the kainate receptor system in gastric acid secretion stimulated by the central injection of agonists for κ-opioid receptors

Glutamate receptor systems are involved in the central stimulation of gastric acid secretion, because central injection of kainate and NMDA stimulated gastric acid secretion (Yang *et al.*, 1993; Kaneko & Taché, 1995; Yoneda & Taché, 1995; Tsuchiya *et al.*, 2001). Although it was reported that kainate and NMDA showed neurotoxic effects by microinjection into several nuclei in rats (Hajnal *et al.*, 1992; Landeira-Fernandez & Grijalva, 1999), we reported that gastric acid secretion induced by the injection of kainate and NMDA at the used doses into the lateral cerebroventricle was not derived from neurotoxicity such as destruction of cells (Tsuchiya *et al.*, 2001). In the present study, it was found that CNQX, an antagonist for non-NMDA receptors such as AMPA and kainate receptors, completely inhibited gastric acid secretion stimulated by the injection of U69593, an agonist for κ_1-opioid receptor, into the lateral cerebroventricle (Figure 1). Treatment with CNQX partially inhibited the secretion stimulated by NBH, an agonist for κ_3-opioid receptor, but had no effect

on the secretion by bremazocine, an agonist for κ_2-opioid receptor. Gastric acid secretion stimulated by dynorphin A-(1-17), which has similar affinity for κ_1–κ_3-opioid receptors (Dhawan *et al.*, 1996; Law & Loh, 1999), was partially inhibited by CNQX. The injection of kainate (0.47 nmol) into the lateral cerebroventricle stimulated the secretion markedly (Figure 5 and Tsuchiya *et al.*, 2001), but the effect of AMPA was limited even at the higher dose (37.4 nmol) (Tsuchiya *et al.*, 2001). Thus, an inhibitory effect of CNQX on the secretion appeared to be derived from inhibition of the kainate receptor. In contrast, treatment with CPP, an antagonist for the NMDA receptor, did not affect gastric acid secretions stimulated by dynorphin A-(1-17) and by the tested selective agonists for κ_1–κ_3-opioid receptors (Figure 2). These findings suggest that (1) among κ-opioid receptors, the κ_1- and κ_3-opioid receptor systems interacted with the kainate, but not the NMDA receptor system, and stimulated gastric acid secretion, and (2) the secretion stimulated by activation of the κ_2-opioid receptor system was independent of the NMDA and kainate receptor systems in the brain regions close to the lateral cerebroventricle in rats (Table 4).

Although the κ-opioid receptor system inhibited glutamatergic transmission and glutamate release in various brain regions (McGinty, 1999; Ogura & Kita, 2000), some investigators showed that activation of central κ-opioid receptors increased the amount of glutamate in the cerebrospinal fluid in rats (Bakshi *et al.*, 1990; Skilling *et al.*, 1992). Kainate receptors, specifically kainate receptor subunits (GluR5-7, KA1 and KA2), are widely distributed in the hypothalamus, including the arcuate nucleus and paraventricular nucleus of the hypothalamus (Van Den Pol *et al.*, 1994; Vignes and Collingridge, 1997; Eyigor *et al.*, 2001). Thus, activation of κ_1- and κ_3-opioid receptors may release glutamate as a following neurotransmitter and thus stimulate gastric acid secretion via activation of kainate receptors in the brain regions close to the lateral cerebroventricle.

Involvement of the $GABA_A$, but not $GABA_B$, receptor system on gastric acid secretion stimulated by the injection of agonists for κ-opioid receptors

GABA and its receptor systems in the CNS are important to stimulate gastric acid secretion (Lin *et al.*, 1988; Del Tacca *et al.*, 1990; Lin, 1995; Watanabe *et al.*, 2000). Bicuculline infusion completely inhibited gastric acid secretion stimulated by the injections of U69593 and bremazocine into the lateral cerebroventricle (Figure 3). Both the secretions stimulated by NBH and dynorphin A-(1-17) were partially inhibited by bicuculline infusion. Interestingly, the secretions in the initial phase during 0–40 min induced by the injection of dynorphin A-(1-17) and NBH were slightly, not significantly, inhibited by treatment with bicuculline, and the secretions in the late phase (during a period of 40–90 min after injections of agonists for κ-opioid receptors) were markedly inhibited by the treatment. The central injection of phaclofen, an antagonist for the $GABA_B$ receptor, did not affect the gastric acid secretion induced by dynorphin A-(1-17) and NBH (Table 2). These findings suggest that (1) the κ_1–κ_3-opioid receptor systems were dependent on activation of the $GABA_A$, not $GABA_B$, receptor system in order to stimulate gastric acid secretion, and (2) activation of κ-opioid receptors by dynorphin A-(1-17), an endogenous agonist, may have been due to the activation of the κ_3-opioid receptor system, mainly in the brain regions close to the lateral cerebroventricle in rats (Table 4). Bicuculline was administrated systemically by intravenous infusion in the present study, and it has been reported that activation of peripheral $GABA_A$ receptors stimulated or potentiated gastric acid secretion in rats (Harty *et al.*, 1991; Lin, 1995). In the present study, however, the $GABA_A$ receptor system functioned centrally, not peripherally, to regulate gastric acid secretion, because gastric acid secretion stimulated by the injection of agonists for κ-opioid receptors into the fourth cerebroventricle was not inhibited by bicuculline treatment (Table 3).

Many pharmacological findings showed that opioids exerted their excitatory action in the brain indirectly, by inhibiting release of the inhibitory neurotransmitter GABA (Mulder & Schoffelmeer, 1993; Sbrenna *et al.*, 1999). Thus, the exact mechanisms for interactions between κ-opioid receptors and GABA receptor systems are unknown. It was reported that κ-opioid receptors present at the presynapses act as the inhibitory regulator of the release of methionine-enkephalin, an endogenous agonist for μ-opioid receptor (Ueda *et al.*, 1987). A contribution of inhibitory neurons activated by the μ-opioid receptor system may decrease and thus stimulate GABA release in the brain regions close to the lateral cerebroventricle. Another possibility is the involvement of neurons, which can release glutamate by the activation of κ-opioid receptors. As described above, several studies reported that the activation of central κ-opioid receptors increased glutamate release in the CNS (Bakshi *et al.*, 1990; Skilling *et al.*, 1992). Recent studies established that activation of kainate receptors enhanced the release of GABA from some brain regions including hippocampal interneurons (Cossart *et al.*, 2001; Jaffe & Figueroa, 2001; Kerchner *et al.*, 2001). In the present study, gastric acid secretion stimulated by the central injection of kainate was inhibited by bicuculline infusion (Figure 5). Thus, the glutamate released by activation of κ-opioid receptors may stimulate GABA release from interneurons via activation of kainate receptors, and thus stimulate gastric acid secretion via activation of $GABA_A$ receptors in the CNS. It has to be determined whether glutamate and GABA are released by activation of κ-opioid receptors in the brain regions close to the lateral cerebroventricle.

Interaction of κ-opioid and other receptor systems in the respective brain regions regulating gastric acid secretion

Although there are differences in the subtypes, the κ-opioid receptor systems appeared to interact with the kainate receptor system and thus stimulate gastric acid secretion in the rat brain regions close to the lateral cerebroventricle (Table 4). In the brain regions close to the fourth cerebroventricle, however, the κ-opioid receptor system appeared not to interact with the kainate receptor system. The injection of CNQX into the fourth cerebroventricle alone, but not into the lateral cerebroventricle, markedly stimulated gastric acid secretion. In addition, the $GABA_A$ receptor system regulated the secretion stimulated by activation of κ-opioid receptors in the brain regions close the lateral, but not the fourth, cerebroventricle. Thus, the interactions between κ-opioid, glutamate and GABA receptor systems are different, depending on each brain region.

It was reported that excitation of the raphe pallidus by microinjection of kainate induced a vagal-dependent stimulation of gastric acid secretion that was mediated by endogenous thyrotropin-releasing hormone in the dorsal vagal complex in rats (Yang *et al.*, 1993). Several studies have reported the interactions between 5-HT and thyrotropin-releasing hormone receptor systems in the medullary caudal raphe nuclei and the dorsal vagal complex affecting gastric acid secretion (Chi *et al.*, 1996; Yang *et al.*, 2000). The bilateral microinjection of ketanserin (1 nmol) into the dorsal vagal complex inhibited gastric acid secretion stimulated by the microinjection of kainate (0.45 nmol) into the raphe pallidus (Yoneda & Taché, 1995). In the brain regions close to the lateral cerebroventricle, however, the 5-$HT_{2/1C}$ receptor systems at least were not involved in the secretion by activation of κ-opioid receptors because ketanserin (5.5 nmol) did not show an inhibitory effect. The interactions between the κ-opioid and other receptor systems should be determined in the respective fine brain regions in future studies.

Summary and evidence for κ-opioid receptor subtypes

The molecular nature of κ_2- and κ_3-opioid receptors is still unclear because only a single κ-opioid receptor clone (κ_1) has been detected within various species (Raynor *et al.*, 1994). The κ_2- and κ_3-opioid receptors may represent post-translational modifications of one gene product (κ_1), or the heterogeneous association of other molecules. Recent findings suggest that the κ_2-opioid receptors are in fact a mixed population of κ_1- and δ- (Jordan & Devi, 1999) or μ-opioid receptors (Simonin *et al.*, 2001). However, much evidence has shown the existence of κ-opioid receptor subtypes, at least pharmacologically (Satoh & Minami, 1995; Dhawan *et al.*, 1996; Law and Loh, 1999; Heyliger *et al.*, 1999). For example, bremazocine (κ_2), but not U69593 (κ_1), caused a decrease in synaptically evoked NMDA receptor-mediated currents and thus produced the antihyperalgesic effect (Ho *et al.*, 1997). A synthesized agent GR89696 acted as an agonist and antagonist for κ_2- and κ_1-opioid receptors, respectively, in the guinea-pig hippocampus (Caudle *et al.*, 1997), and showed an agonist profile consistent with an action through κ_2-opioid receptors *in vitro* and *in vivo* in rhesus monkeys (Butelman *et al.*, 2001). Pan *et al.* (1995) reported that there was a clone of the κ_3-related opioid receptor in the mouse brain, although it is still unclear whether the clone is the κ_3-opioid receptor itself.

The present findings suggest the existence of three pharmacologically different subtypes of κ-opioid receptors, which are all positively coupled to gastric acid secretion, having different interactions with other receptor systems, in the brain regions close to the lateral cerebroventricle in rats: (1) the κ_1-opioid receptors largely interacted with kainate and $GABA_A$ receptors; (2) the κ_2-opioid receptors interacted with $GABA_A$, but not with kainate, receptors; and (3) the κ_3-opioid receptors partially, but not significantly, interacted with kainate and $GABA_A$ receptors. A cloning of each subtype and more biochemical characterization are necessary to support the proposed subtypes of κ-opioid receptors.

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