Differential Effects of $\mu$-, $\delta$-, and $\kappa$-Opioid Receptor Agonists on Mechanosensitive Gastric Vagal Afferent Fibers in the Rat

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Ozaki, Noriuki, J. N. Sengupta, and G. F. Gebhart. Differential effects of $\mu$-, $\delta$-, and $\kappa$-opioid receptor agonists on mechanosensitive gastric vagal afferent fibers in the rat. J. Neurophysiol. 83: 2209–2216, 2000. Single-fiber recordings were made from the decentralized right cervical vagus nerve (hyponodosal) of the rat. A total of 56 afferent fibers that responded to gastric distension (GD) were studied: 6 fibers were stimulated by phasic balloon GD, 50 by fluid GD. All fibers gave increasing responses to increasing pressures of GD (5–60 mmHg). The effects of $\mu$-opioid (morphine), $\delta$-opioid (SNC80), and $\kappa$-opioid (EMD61,753, U62,066) receptor agonists were tested on responses of afferent fibers to GD. Morphine, administered systemically over a broad dose range (10 $\mu$g to 31 mg/kg, cumulative), had no effect on either resting activity or responses of vagal afferent fibers to GD. Similarly, the $\delta$-opioid receptor agonist SNC80 (0.05–3.2 mg/kg) did not affect resting activity or responses to GD. In contrast, cumulative intra-arterial doses of the $\kappa$-opioid receptor agonist EMD61,753 or U62,066 dose dependently attenuated afferent fiber responses to GD. Doses producing inhibition to 50% of the control response to GD of EMD61,753 (8.0 mg/kg) and U62,066 (8.8 mg/kg) did not differ. The effect of U62,066 was moderately attenuated by a nonselective dose (4 mg/kg) of naloxone hydrochloride; the $\kappa$-opioid receptor-selective antagonist nor-BNI (20 mg/kg) was ineffective. These results demonstrate that $\kappa$-, but not $\mu$- or $\delta$-opioid receptor agonists modulate visceral sensation conveyed by vagal afferent fibers innervating the stomach. Given that $\kappa$-opioid receptor agonists effects were only modestly antagonized by naloxone and not at all by nor-BNI, the results point to a novel site of action.

INTRODUCTION

The antinociceptive effects of opioids are mediated through activation of opioid receptors in central and peripheral tissues. To date, three opioid receptors ($\mu$, $\delta$, and $\kappa$) have been shown to be involved in the modulation of visceral nociception. In a model of visceral nociception in the rat, intrathecal administration of $\mu$- and $\delta$-opioid receptor agonists (ORAs), but not the $\kappa$-ORA U50,488, attenuate visceromotor and cardiovascular responses to noxious colorectal distension (Dunzebrink et al. 1995; Diop et al. 1994; Harada et al. 1995; Ness and Gebhart 1988). Systemic administration of $\kappa$-ORAs, however, dose dependently attenuates responses to noxious colorectal distension (Burton and Gebhart 1998). Electrophysiological studies resolved that in addition to a supraspinal site of action, $\kappa$-ORAs have direct peripheral actions as well. We found that putative $\kappa$- (U50,488, U69,593, U62,066), $\kappa_2^\prime$ (bremazocine), and $\kappa_3^\prime$ (naloxone benzoylhydrazide) ORAs, a peripherally restricted $\kappa$-ORA (EMD61,753), and a mixed $\kappa/\mu$-ORA (fedotozine), but not either $\mu$- (morphine and fentanyl) or $\delta$- (4-phenyl-

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stripped at the tips were placed around the nerve and sealed with non-reactive Wacker gel (Wacker Silicone, Adrian, MI).

For phasic balloon gastric distension (GD), a 2.0–2.5 cm long, 2–3 cm diam flaccid, flexible latex balloon was placed surgically in the stomach through the fundus. The balloon occupied approximately two-thirds of the proximal stomach. The pylorus was not obstructed, and there was no blockage of gastric emptying. The outside diameter of the balloon when inflated was greater than the intraluminal diameter of the stomach of the rat. Therefore the pressure measured during GD reflected actual intragastric pressure. The balloon catheter was connected to a distension control device via a low volume pressure transducer (see Gebhart and Sengupta 1996 for details).

For fluid GD, the stomach was intubated with flexible Tygon tubing (2.3 mm OD, 1.3 mm ID) via the mouth, esophagus, and cardia. The catheter was secured by a ligature around the esophageal-gastric junction. Another Tygon tube (3.9 mm OD, 2.4 mm ID) was introduced distally through the pylorus and was secured by a ligature placed caudal to the pyloric sphincter; the duodenum was ligated close to the pyloric ring. For GD, the oral catheter was connected to a reservoir containing saline at room temperature. Constant pressure distension was achieved using the distension control device with the distal catheter clamped. Intragastric pressure was monitored by connecting the distal catheter via a three-way stopcock to a low-volume pressure transducer. The abdomen was closed with silk sutures.

Recording of afferent nerve activity

The right vagus nerve was exposed by a ventral midline incision in the neck. The sternocleidomastoid, sternohyoid, and omohyoid muscles were removed. The skin was reflected laterally and tied to the stereotaxic frame to make a pool for warm mineral oil (37°C). The nerve was dissected away from the carotid tissue sheath, decentralized close to its entry to the nodose ganglion, and placed over a black micro-base plate. The perineural sheath was removed in the pool of warm mineral oil, the nerve was split into thin bundles, and fine filaments were teased from the bundle to obtain a single unit. Electrical activity of the single unit was recorded by placing the fiber over one arm of a bipolar silver-silver chloride electrode.

A fine strand of connective tissue was placed over the other pole of the electrode for differential recording.

Action potentials were monitored continuously by analogue delay and displayed on a storage oscilloscope after low noise AC differential amplification. Action potentials were processed through a window discriminator and counted (1 s binwidth) on-line using the SPIKE2/CED 1401 data acquisition program (Cambridge Electronic Design, Cambridge, UK). Peristimulus time histograms, intragastric pressure, and blood pressure were displayed on-line continuously. Data were also recorded on tape for later analysis.

Experimental protocol

Mechanosensitive gastric muscle afferents in the vagus nerve were identified by response to a test stimulus of GD (40 mmHg, <5 s). If a fiber responded to GD, a stimulus-response function (SRF) to distending pressures of 5, 10, 20, 30, 40, and 60 mmHg, 30 or 60 s duration at 4-min intervals was determined.

To measure conduction velocity, the vagus nerve was stimulated with a single 0.5-millisecond square-wave pulse at 3–8 mA, and the conduction delay (time between stimulus artifact and evoked response) was recorded. The conduction distance was measured postmortem. Fibers were classified on the basis of their conduction velocities; those with conduction velocities <2.5 m/s were considered unmyelinated C-fibers, and those with conduction velocities >2.5 m/s were considered thinly myelinated Aδ-fibers.

The effects of representative μ- (morphine), δ- (SNC80), and κ- (EMD61,753, U62,066) ORAs were tested on resting activity and responses to 60 mmHg GD (30 or 60 s, every 4 min) of mechanosensitive gastric afferent fibers. All drugs were administered intra-arterially in a cumulative dose paradigm. Each dose of drug was given 120 s before the onset of distension. Cumulative dose-response relationships for morphine were obtained by giving cumulative doses of 0.5, 1, 2, 4, and 8 mg/kg; doses of SNC80 were 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/kg. Cumulative doses of EMD61,753 and U62,066 were 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, and 32.0 mg/kg. Thirteen fibers that were not affected by morphine were subsequently tested in the presence of other ORAs.

To determine whether effects of κ-ORAs were produced at opioid

![FIG. 1. Responses of vagal afferent fibers to graded intensities (5–60 mmHg) of gastric distension (GD). Responses are illustrated topmost in each record as peristimulus time histograms (1-s binwidth); intragastric pressure is presented below. From top to bottom; responses of a fiber to 30-s balloon GD, responses of a fiber to 30-s fluid GD, and responses of a fiber to 60-s fluid GD.](http://jn.physiology.org/Downloadedfrom)
receptors, the effects of U62,066 (8 mg/kg) alone \((n = 4)\) and after intra-arterial administration of a non-receptor-selective dose of naloxone hydrochloride (NLXH, 4 mg/kg; \(n = 5\)) on response to GD were examined. The effects of U62,066 (8 mg/kg) were also tested before and after administration of NLXH in three fibers. The effects of U62,066 were studied in the presence of nor-binaltorphimine dihydrochloride (nor-BNI), a selective \(\kappa\)-opioid receptor antagonist. Nor-BNI (20 mg/kg sc) was injected 24 h before \((n = 4)\) or 24 and 48 h before experiments \((n = 1)\).

At the end of the protocol for each fiber, the abdomen was opened and the mechanosensitive receptive field was located by probing the stomach with a fine, blunt glass rod.

**Drugs**

Morphine sulfate (MW: 668.7, Merck Chemical Division, Merck, Rahway, NJ), U62,066 (MW: 356.5, Research Biochemicals, Natick, MA), naloxone hydrochloride (MW: 363.8, Sigma Chemical, St. Louis, MO), and nor-binaltorphimine dihydrochloride (nor-BNI; MW: 698.27, Tocris Cookson, St. Louis, MO) were dissolved in 0.9% saline. SNC80 (MW: 449.6, Tocris Cookson) was dissolved in 10% DMSO and 1% acetic acid. EMD61,753 (MW: 469.1, E. Merck, Darmstadt, Germany) was dissolved in 10% DMSO.

**Data analysis**

The resting activity of a fiber was counted for 60 s before GD, and the response to GD was determined as the increase in discharge during GD above its resting activity \((\text{imp/s})\). SRFs to graded GD were plotted for each individual fiber, and a least-squares regression line was obtained from the linear part of the SRF. The regression line then was extrapolated to the ordinate (representing distension pressure) to estimate response threshold.

All data are expressed as means ± SE. Results were analyzed using paired or unpaired Student’s \(t\)-test. The inhibitory dose 50 (ID\(_{50}\); dose to produce 50% inhibition of the response to distension) and 95% confidence intervals were calculated from the 20–80% component of the cumulative dose-response curve (Tallarida and Murray 1987). A value of \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Fiber sample**

A total of 56 gastric afferent fibers were studied, all of which responded to GD. In the presence of the balloon in the stomach \((n = 6)\), fibers exhibited ongoing activity \((\text{mean: } 3.9 ± 2.2 \text{ imp/s}; \text{range: } 0.02–14.2 \text{ imp/s})\). Forty-two of 45 fibers that responded to fluid GD were spontaneously active \((\text{mean: } 1.5 ± 0.3 \text{ imp/s}; \text{range: } 0.01–8.5 \text{ imp/s})\). Five of the total 56 fibers were pretreated 24 or 48 h before an experiment with nor-BNI \((n = 5); \text{mean spontaneous activity: } 3.2 ± 1.4 \text{ imp/s}; \text{range: } 0.2–8.5 \text{ imp/s})\).

Conduction velocities of 15 fibers that responded to fluid GD were measured by electrical stimulation of the vagus nerve. All 15 fibers were unmyelinated C-fibers \((\text{mean conduction velocity: } 0.6 ± 0.02 \text{ m/s}; \text{range: } 0.5–0.9 \text{ m/s})\). In a previous study of gastric vagal afferent fibers (Ozaki et al. 1999), we also encountered only C-fibers \((n = 27); \text{mean conduction velocity: } 0.7 ± 0.06 \text{ m/s})\).

**SRFs**

Responses to graded balloon or fluid GD were studied in all 56 fibers \((\text{see Fig. 1 for examples})\). SRFs of fibers in the different experimental groups, excepting five fibers from rats pretreated with nor-BNI, are given in Fig. 2. Extrapolation of the linear portion of individual SRFs revealed that gastric vagal afferent fibers exhibited a range of thresholds for response to GD. The mean response threshold to 30 s balloon GD was 7.9 ± 1.6 mmHg \((\text{range: } 2.0–13.8 \text{ mmHg})\), to 30 s fluid GD 3.8 ± 1.2 mmHg \((\text{range: } 0–16.9 \text{ mmHg})\), and to 60 s fluid GD 4.4 ± 0.6 mmHg \((\text{range: } 0–12.6 \text{ mmHg})\). This sample of 51 fibers is very similar to a different sample of vagal afferent fiber using the same methods to distend the stomach (Ozaki et al. 1999).

The mean SRFs of vagal afferent fibers to GD are presented in Fig. 2D. When responses to the more natural fluid filling of the stomach were compared with responses to phasic balloon GD, response magnitude generally was greater to fluid GD, particularly during the second half of the period of distension. Although GD was delivered at a constant pressure whether by balloon or fluid, the rate at which the final pressure was achieved and the duration of the target pressure differed for the three groups.

**FIG. 2.** Stimulus response functions (SRFs) of 51 gastric vagal afferent fibers to graded intensities of GD. A: responses to 30-s balloon GD. B: responses to 30-s fluid GD. C: responses to 60-s fluid GD. D: mean SRFs for the 3 different groups.
two types of GD (see pressure traces in Fig. 1). The mean SRF of vagal afferent fibers to balloon GD significantly differed from the SRFs to fluid GD. Characteristics of this sample of gastric vagal afferent fibers are similar to what we found in an earlier study (Ozaki et al. 1999).

Effects of opioid receptor agonists (ORAs) on gastric mechanosensitive vagal afferent fibers

**μ-ORA.** When we began these studies, we tested morphine first and anticipated that relatively low concentrations would affect vagal afferent fiber activity (e.g., Randich et al. 1991). We tested cumulative doses of morphine from 10–300 μg/kg (n = 11), progressing in subsequent experiments to a cumulative dose of 31 mg/kg. A total of 19 fibers were studied and spontaneous activity was not affected by morphine.

The mechanosensitive properties of 13 fibers were tested 10 min after injection of cumulative doses of morphine (10–440 μg/kg, n = 7; 10–1,440 μg/kg, n = 1; 1–15 mg/kg, n = 4; 1–31 mg/kg, n = 1). Responses to GD did not change at any of the pressures of GD tested (5–60 mmHg) at any dose of morphine.

**FIG. 3.** Example of responses of a gastric vagal afferent fiber to graded intensities of fluid gastric distension (GD; 60 s) before and 10 min after injection of a 1,440-μg/kg dose of morphine into the left carotid artery. Responses are illustrated as peri-stimulus time histograms (1-s binwidth); intragastric pressure is given below. **Inset:** summary of stimulus response functions of 13 fibers to graded balloon GD (30 s), fluid GD (30 s), and fluid GD (60 s) before and 10 min after different doses of morphine (440 μg/kg, 1,440 μg/kg, 15 mg/kg, and 31 mg/kg).

**FIG. 4.** Examples of lack of effect of μ- (morphine) and δ- (SNC80) opioid-receptor agonists on responses of gastric vagal afferent fibers to repeated fluid GD (60 mmHg, 30 s every 4 min). Responses are illustrated as peristimulus time histograms (1-s binwidth); intragastric pressure is presented below. Drugs were injected intra-arterially in a cumulative dose as indicated (†).
morphine. Figure 3 shows an example of responses of an afferent fiber to graded GD before and after injection of morphine; the inset illustrates the mean SRFs of all 13 fibers before and after various doses of morphine.

In previous studies of pelvic nerve afferent fibers, we tested cumulative doses of ORAs on a single, repeated distending pressure. Accordingly, we tested the effects of cumulative doses of morphine (8 mg/kg) on responses of five fibers to repeated GD (60 mmHg, 60 s). Figure 4 illustrates the absence of effect of morphine on responses of a gastric vagal afferent fiber to distension. The data are summarized in Fig. 5.

**DISCUSSION**

The present results document an inhibitory effect of κ-ORAs on responses of gastric vagal afferent fibers to GD. Kappa ORAs, but neither morphine (μ-ORA) nor SNC80 (δ-ORA), dose dependently attenuated responses to GD. This is consistent with previous studies of ORA effects on pelvic nerve afferent fiber responses to urinary bladder (Su et al. 1997a) or colorectal distension (Sengupta et al. 1996, 1999; Su et al. 1997b). In the present study, as in previous studies, naloxone was able to antagonize in part the effects of the κ-ORAs tested, whereas the κ-opioid receptor antagonist nor-BNI was without effect. We interpreted this outcome to suggest that their exists in the viscer a opioid-like receptor at which κ-ORAs act to attenuate visceral nociception, and the present results are consistent with this interpretation.

Differential effects of κ-ORAs in binding, physiological, and behavioral studies suggest the existence of three κ-opioid receptors (κ1, κ2, and κ3). Benzacetamide κ-ORAs (U50,488, U62,066, EMD67,753) bind preferentially to a κ1-opioid receptor, whereas benzomorphan compounds (bremazocine and ethylketocyclazocine) bind preferentially to κ2-opioid receptors (Caudle et al. 1998; Clark et al. 1989; Zukin et al. 1988). Behavioral characterization of κ1 and κ2 receptors in a rat model of persistent pain suggested that κ1-ORAs are ineffective as analgesics when injected intracereally, whereas agonists that have high affinity for κ2 receptors are effective intrathe-
cally in modulating hyperalgesia and allodynia (Ho et al. 1997). This is consistent with the recent report by Ness (1999) showing that intrathecally administered U50,488, a $\kappa_1$-selective agonist had no effect on spinal neurons excited by colorectal distension. Radioligand binding studies indicate that in the spinal cord of rats, guinea pig, monkeys, and humans $\kappa_2$ receptors are 10-fold more abundant than $\kappa_1$ receptors (Caudle et al. 1998). The existence of functional $\kappa_2$ receptors requires molecular evidence, which is presently not available. It has been proposed that this receptor could be a posttranslational modification of the cloned $\kappa_1$ receptor (Caudle et al. 1998). Radioligand binding and antisense studies also indicate the existence of two affinity sites of $\kappa_1$ receptors for $\kappa$-ORAs; $\kappa_{1a}$ and $\kappa_{1b}$ (Clark et al. 1989; Lai et al. 1994; Pasternak et al. 1999; Rothman et al. 1990).

Support for presence in the viscera of a $\kappa$-like receptor different from the $\kappa_1$-opioid receptor cloned in the CNS is based on the following. First, the mean effective doses of the two $\kappa$-ORAs studied here that attenuated responses of gastric vagal afferent fibers to GD (8.0 mg/kg for EMD61,753; 8.8 mg/kg for U62,066) are not different from each other or different from doses of the same and other $\kappa$-ORAs that attenuated responses of pelvic nerve afferent fibers to colorectal or urinary bladder distension to 50% of control (Sengupta et al. 1996; Su et al. 1997a,b). These results are in contrast to the >100-fold differences reported in the literature for these same $\kappa$-ORAs in binding studies (Clark et al. 1989; Devlin and Shoemaker 1990; Rothman et al. 1990; Zukin et al. 1988) and various models of nociception. Effective antinociceptive doses of $\kappa$-ORAs have been reported to range from the $\mu$g/kg to mg/kg range (e.g., Burton and Gebhart 1998; Herrero and Headley 1993; Hunter et al. 1990; Paul et al. 1990). In unanesthetized, intact rats, where $\kappa$-ORAs have access to both peripheral and central sites of action, effective doses of $\kappa$-ORAs for 50% attenuation of visceromotor or pressor responses to colorectal distension range from 10 $\mu$g/kg to 8 mg/kg (Burton and Gebhart 1998). Second, the $\kappa$-selective opioid receptor antagonist nor-BNI was without effect in the present study. Nor-BNI can have a long latency to effect and has a long duration of action (Spanagel et al. 1994; Takemori et al. 1988). Accordingly, we pretreated rats for 48 or 24 h with nor-BNI, but did not observe any attenuation of the effect of the $\kappa$-ORAs tested. Naloxone, moreover, was only modestly effective in partly attenuating the effects of U62,066 on responses of gastric vagal afferent fibers to GD. We have considered previously that the $\kappa$-like receptor in the viscera may be an orphan receptor similar to opioid-receptor–like 1 (ORL1), at which the endogenous orphanin FQ/nociceptin peptide acts (Meunier et al. 1995; Reinscheid et al. 1995). The ORL1 receptor most closely resembles the $\kappa$-opioid receptor, although it is distinct from the $\kappa$- and other opioid receptors (Meunier 1997). We tested nociceptin on responses of pelvic nerve afferent fibers to colonic distension and observed no effect (V. Julia and G. F. Gebhart, unpublished observations). The absence of antagonism of effects by nor-BNI and modest antagonism by naloxone suggests that effects were produced at a nonopioid receptor.

Benzacetamide $\kappa$-ORAs have been reported to exert local anesthetic-like effects on Na$^+$ channels (Alzheimer and Ten Bruggencate 1990; Pugsley and Goldin 1999; Zhu and Im 1992; Zhu et al. 1992). In vitro studies reveal that benzacetamide $\kappa$-ORAs as well as benzacetamide compounds with very poor binding affinity for $\kappa$-opioid receptors produce concentration-dependent blockage of Na$^+$ channels (e.g., Pugsley and Goldin 1999; Pugsley et al. 1993). Structurally related compounds have also been shown to possess anticonvulsant and antiarrhythmic efficacy (Pugsley et al. 1992, 1993; Zhu et al. 1992). The $\kappa$-ORAs studied here did not affect conduction velocity of pelvic nerve afferent fibers or action potential amplitude (Sengupta et al. 1996; Su et al. 1997a,b), suggesting the absence of a local anesthetic-like effect. Moreover, $\kappa$-ORAs effects on mean arterial pressure in the present experiments

![Diagram](http://jn.physiology.org/10.2220.32.246)
were modest (≤10 mmHg decrease) compared with the magnitude of the nonopioid receptor-mediated hypotension reported in similarly barbiturate-anesthetized rats (Pugsley et al. 1992, 1993). Moreover, the μ-, δ-, and κ-ORs tested here produced equivalent, modest hypotensive effects. Although these considerations suggest that a local anesthetic-like action does not explain the current results, we cannot exclude this possibility based on the data collected.

We were surprised that morphine, studied over a very broad dose range, did not affect either resting activity or responses of gastric vagal afferent fibers to GD. There is a considerable literature that suggests that morphine (0.1–2.5 mg/kg iv) and other μ-ORs activate vagal afferent fibers and in so doing contribute to analgesia. For example, Randich et al. (1991) reported that bilateral cervical vagotomy significantly attenuated the antinociception produced by morphine given intravenously. The effects were dose dependent, and the role of vagal afferent fibers was greatest at the lower doses of morphine tested (see also Randich and Gebhart 1992). With respect to the viscera, Kumazawa et al. (1989) reported that morphine generally excited spermatic nerve afferent fibers in vitro. In a study in anesthetized cats, Balkowiec et al. (1994) applied morphine to the receptive fields of thoracic viscera and noted that 7 of 10 vagal afferent fibers tested increased activity. Eastwood and Grundy (1995) reported that morphine, the μ-receptor–selective peptide agonist [D-Ala²,N-Me-Phe⁴,Gly⁵-ol]-enkephalin (DAMGO) and the δ-receptor-selective agonist [D-Ala²,Leu⁵]-enkephalin (DADLE), but not U50,488, stimulated both vagal and nonvagal intestinal afferents in the rat. Excitatory effects of opioid agonists have also been described in vitro. Very low concentrations of μ-, δ-, and κ-selective opioid agonists decrease a voltage-activated K⁺ current in mouse dorsal root ganglion (DRG) neurons in culture (Fan et al. 1993). Furthermore, nanomolar concentrations of μ-, δ-, and κ-selective agonists prolong the action potential duration in DRG neurons (Crain and Shen 1990; Shen and Crain 1989, 1994a,b).

There have been relatively few studies of κ-OR effects on single afferent fibers (not including those from this laboratory). In a sample of articular afferent fibers innervating the inflamed knee joint of the cat, Russell et al. (1987) reported that two κ-ORAs (U50,488 and ethylketocyclazocine) significantly depressed spontaneous activity in a naloxone-reversible manner. Andreev et al. (1994) examined the effects of the κ-ORA U69,593 on polymodal nociceptors in the saphenous nerve using dynorphin A 1–13 and U69,593 in the rat brain. Clark, J. A., Liu, L., Price, M., Hersh, B., Edelson, M., and Pasternak, G. W. Kappa opiate receptor multiplicity: evidence for two U50,488-sensitive κ₁ subtypes and a novel κ₂ subtype. J. Pharmacol. Exp. Ther. 251: 461–468, 1989.


