Sodium Channel Blocking Actions of the κ-Opioid Receptor Agonist U50,488 Contribute to Its Visceral Antinociceptive Effects

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Su, X., S. K. Joshi, S. Kardos, and G. F. Gebhart. Sodium channel blocking actions of the κ-opioid receptor agonist U50,488 contribute to its visceral antinociceptive effects. J Neurophysiol 87: 1271–1279, 2002; 10.1152/jn.00624.2001. The goal of the present study was to determine whether the κ-opioid receptor agonist (ORα) U50,488 attenuates behavioral and primary afferent nerve responses to noxious colorectal distension (CRD) by sodium channel blockade. We tested the analgesic κ-ORα (±)-trans U50,488, its enantiomers (−)-trans (1S,2S)-U50,488 and non κ-ORα (−)-trans (1R,2R)-U50,488, and/or its diastereomer (−)-cis (1S,2R)-U50,488 for their ability to attenuate visceromotor and pelvic nerve afferent fiber responses to noxious CRD in vivo and voltage-activated sodium current in colon sensory neurons in vitro. In unanesthetized rats, subcutaneous administration of U50,488, (1S,2S)-U50,488, and (1R,2R)-U50,488 attenuated the behavioral visceromotor response to noxious CRD; the rank order of potency was: (1S,2S)-U50,488 > U50,488 ≫ (1R,2R)-U50,488. U50,488 and its stereoisomers also inhibited responses of decentralized pelvic nerve afferent fibers to noxious CRD in a dose-dependent manner. Cumulative doses of 16 mg/kg of (1S,2S)-U50,488, (1S,2R)-U50,488, and (1R,2R)-U50,488 reduced responses to a mean 29, 30, and 47% of control, respectively. The mean inhibitory doses of these drugs were not different (range: 6.6–10.8 mg/kg). Sodium channel blockers mexiletine and carbamazepine mimicked the effect of U50,488. In contrast, the κ-ORαs dynorphin (1–13) and ICI 204,488 were ineffective in attenuating pelvic nerve activity. Perfusion of (1S,2S)-U50,488, (1S,2R)-U50,488, or (1R,2R)-U50,488 on colon sensory neurons in vitro decreased voltage-activated sodium currents. This inhibition by U50,488 and its stereoisomers was not opioid receptor-mediated because it could not be reversed by the opioid receptor antagonist naloxone and was also not a G protein–mediated effect. The results reported here suggest that the visceral antinociceptive effects of U50,488 and its stereoisomers are contributed to by their peripheral sodium channel blocking actions.

INTRODUCTION

The mechanisms and modulation of visceral pain have been extensively examined using colorectal distension (CRD) as a noxious visceral stimulus. Previous studies employing CRD have documented the ability of κ-opioid receptor agonists (ORαs) like U50,488 to attenuate pseudoadaptive visceromotor and cardiovascular responses to noxious CRD following their systemic, but not intrathecal administration (Danzebrink et al. 1995; Harada et al. 1995a,b). A peripheral, visceral antinociceptive action of U50,488 has been demonstrated. U50,488 was shown to dose dependently inhibit responses of mechanosensitive pelvic nerve afferent fibers to noxious colorectal or urinary bladder distension in the rat (Sengupta et al. 1996; Su et al. 1997a,b). This inhibition could not be completely antagonized by very high doses of naloxone or by two κ-opioid receptor-selective antagonists, nor-Binaltorphimine dihydrochloride (nor-BNI) and DIPPA. Furthermore, in experiments where the cloned rat κ-opioid receptor (KOR) 1 was “knocked-down” at peripheral sites using antisense oligodeoxynucleotides, the dose-dependent inhibition of pelvic nerve afferent fiber responses to noxious CRD by κ-ORαs persisted (Joshi et al. 2000). These results suggested that these peripheral, visceral κ-ORα actions are mediated by a nonopioid mechanism. Additional electrophysiological studies have demonstrated the nonopioid-dependent ability of arylbenzacetamide κ-ORαs like U50,488 to block voltage-activated sodium currents in hippocampal CA3 neurons (Alzheimer and Bruggencate 1990) and cardiac myocytes (Pugsley et al. 1993, 1994).

The synthesis of optically pure stereoisomers of the traditionally used analgesic κ-ORα U50,488 [i.e., (±)-trans U50,488] as well as their in vitro receptor selectivities and pharmacological activities have been described (Pugsley et al. 1993; Rothman et al. 1989; Zhu and Im 1992). Due to their structural similarity but distinct opioid pharmacology, U50,488 and its stereoisomers are a valuable tool to examine whether their particular pharmacological action involves an opioid mechanism or otherwise.

Accordingly, the objective of the present study was to test the hypothesis that the peripheral visceral antinociceptive effect of U50,488 and κ-ORαs is contributed to by an effect at voltage-activated sodium channels. This was done by testing the ability of U50,488 and its enantiomers to attenuate visceromotor responses and inhibit responses of mechanosensitive pelvic nerve afferent fibers to noxious CRD. We also compared the effects of U50,488 and its stereoisomers with those of the κ-ORα peptide dynorphin A (1–13) and a structurally-modified arylbenzacetamide κ-ORα, ICI 204,488, and with the sodium channel blockers mexiletine and carbamazepine. We then examined in preliminary experiments the effects of U50,488 and its stereoisomers on voltage-activated sodium currents in colon sensory neurons.

METHODS

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed 1–2 per cage with free access to food and water and were maintained

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on a 12-h light-dark cycle (lights on 06.00–18.00 h) in the Association for Assessment and Accreditation of Laboratory Animal Care approved animal care facility. All experimental procedures were approved by the Institutional Animal Care and Use Committee, The University of Iowa.

Behavioral study

CRD in awake rats results in contraction of the abdominal and hindlimb musculature, termed the visceromotor response (VMR). The VMR was quantified by measuring electromyographic (EMG) activity in the external oblique musculature (see Ness and Gebhart 1988 and Gebhart and Sengupta 1996 for details). Teflon-coated stainless steel wire electrodes (Cooner Wires, Chatworth, CA) were stiched into the external oblique musculature immediately superior to the inguinal ligament for EMG recording and externalized at the back of the neck. Rats were allowed to recover for at least 3 days prior to testing.

For CRD, a 6- to 8-cm latex balloon tied to Tygon tubing was inserted intra-anally into the descending colon and rectum. The balloon catheter was connected to a distension control device (see Gebhart and Sengupta 1996) via a low-volume pressure transducer, and intracolonic pressure was continuously monitored. Each distension trial was performed by initiating a constant pressure phasic stimulus (80 mmHg) lasting 20 s. EMG activity was quantified during the 10 s before distension (basal conditions), 20 s of distension, and 10 s following the termination of distension. The EMG signal was amplified (×10,000, 300–5,000 Hz), filtered (200-Hz high pass, 4-pole Butterworth; graphic equalizer, Yamaha), digitized at 500 Hz (DT2800, Data Translation, Marlboro, MA), rectified, and averaged over 500 ms, reducing the effective sampling to 2 Hz. A voltage threshold was arbitrarily set such that few potentials exceeded it under basal conditions. During distension, an increase in EMG activity resulted in an increased number of spikes crossing this preset voltage threshold and were counted using Spike 2 (version 3.18, Cambridge Electronic Design Limited, Cambridge, UK).

The baseline VMR to CRD before drug administration was established by averaging responses to three distensions (80 mmHg, 20 s) given at 4-min intervals. U50,488 compounds or saline were then administered subcutaneously, and responses to CRD were recorded at 10-min intervals for 60 min. Separate groups of rats were also pretreated with the κ-opioid receptor antagonist nor-BNI (10 mg/kg, 24 h, and 0.5 mg/kg, 4 h, prior to testing). All drugs were dissolved in saline for these experiments and were administered in a volume of 1 ml.

The VMR to CRD is presented as percentage of control (% control), where the baseline prior to administration of U50,488 compounds is defined as 100%. The effect of U50,488 was considered statistically significant if the AUC was zero. AUC data were analyzed by a one-way ANOVA followed by a Fisher’s post hoc test; P < 0.05 was considered statistically significant. The inhibitory dose 50 (ID50; 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 1991).

Patch-clamp recording

Di-I (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine methane-sulfonate)-labeled colon sensory neurons in dorsal root ganglia (DRG), identified by their red-orange color in fluorescent light with a Rhodamine filter (excitation wavelength ~546 nM and barrier filter at 580 nM) were selected for study (see Su et al. 1999 for details). The descending colon was exposed, and multiple injections (70 μl total) of Di-I were made using a fine-tipped pipette. The surgical incision was closed, and the animals were allowed to recover 1–2 wk to allow the dye to be transferred to the cell soma of colon sensory neurons in the DRG. The rats were anesthetized, and the S1 DRG were removed. The ganglia tissue was digested in modified Ham’s F-12 culture media containing collagenase (1 mg/ml), trypsin (1 mg/ml), and DNase (0.1 mg/ml) at 37°C for 50 min. The chemical digestion was terminated by adding soybean trypsin inhibitor (2 mg/ml) and bovine serum albumin (1 mg/ml). DRG neurons were resuspended in the modified Ham’s F-12 media supplemented with 5% rat serum and 2% chick embryo extract and plated onto poly-d-lysine–coated glass coverslips. Neurons in the culture media were kept at 37°C in an incubator saturated with water vapor and 5% CO2 and studied within 24 h.

Cells on the coverslips were transferred into a 1-ml recording chamber with medium of the following composition (in mM): 20 NaCl, 70 CholineCl, 40 TEACl, 3.0 MgCl2, 10 HEPES, and 5.5 d-glucose. The pH was adjusted to 7.35 with CsOH, and the osmolality was adjusted to 310 mOsmol/l with sucrose. The patch electrode solution contained (in mM) 5 Na glutamate, 115 CsCl, 2.3 CaCl2, 4.8 MgCl2, 10 EGTA, 10 HEPES, 4.0 Mg-ATP, and 0.5 Na-GTP. The pH was adjusted to 7.25, and the osmolality was adjusted to 310 mOsmol/l. In some experiments, GDP-β-S (1 mM) was used to replace Na-GTP (0.5 mM) in the electrode solution to test for a G protein–mediated effect. After establishing a whole cell recording, linear capacitance was minimized by the analog compensation facility available on the recording amplifier (AOPATCH 200B, Axon Instruments). Series resistance compensation was >80% in all experiments resulting in a final series resistance averaging 4 MΩ. Signals were low-pass filtered at 5 kHz, digitized at 200 μs per point, and stored on a computer for later analysis. Voltage protocols were generated and data acquired and analyzed using pClamp software (version 8.0, Axon Instruments). All data are expressed as means ± SE.
Reduced peak currents or currents at a single test potential were analyzed using Student’s t-test. Current-voltage curves before and after drugs were analyzed by an ANOVA for repeated measures; \( P < 0.05 \) was considered statistically significant.

**Results**

**Behavioral study**

When given subcutaneously, U50,488, (1S,2S)-U50,488, and (1R,2R)-U50,488 attenuated the visceromotor response (VMR) to noxious CRD (80 mmHg, 20 s) in a dose-dependent manner (Fig. 1). The individual ID\(_{50}\) values for U50,488 and (1S,2S)-U50,488 were 4.7 mg/kg (1–23.4 mg/kg) and 0.9 mg/kg (0.4–1.7 mg/kg), respectively. The ID\(_{50}\) value of (1R,2R)-U50,488 was not calculated because the highest dose of the drug tested (100 mg/kg) failed to decrease the VMR to \( \leq 50\% \) of baseline. Thus the rank order of potency of the drugs tested was as follows: (1S,2S)-50,488 > U50,488 > (1R,2R)-U50,488 (Fig. 1). Separate groups of animals were pretreated with the \( \kappa \)-opioid receptor antagonist nor-BNI (10 mg/kg 24 h before and 0.5 mg/kg 4 h before testing) to test the blockade of the maximum effect produced by a given dose of the three compounds. Nor-BNI significantly antagonized the effects of 5 mg/kg (1S,2S)-U50,488 (\( P < 0.05 \)), but not 30 mg/kg (1R,2R)-U50,488 or 10 mg/kg U50,488, consistent with previously reported observations (Burton and Gebhart 1998) (Fig. 1C).

**Single fiber recording**

A total of 48 afferent fibers, 9 A\( \delta \)-fibers (mean CV: 4.9 ± 1.2 m/s, mean ± SE) and 39 C-fibers (mean CV: 2.0 ± 0.2 m/s), that responded to noxious CRD (80 mmHg) in the S1 dorsal root were studied.

**Effects of stereoisomers of U50,488**

All stereoisomers of U50,488 dose-dependently inhibited responses of mechanosensitive pelvic nerve afferent fibers to noxious CRD (\( P < 0.05 \)); examples are given in Fig. 2, and summary data are presented in Fig. 3A. All stereoisomers also dose-dependently reduced heart rate (\( P < 0.05 \); summarized in Fig. 4). The slopes of the dose-regression functions and the doses producing inhibition to 50\% of the control response to 80 mmHg CRD did not differ among the three stereoisomers (Table 1).

We have previously documented that the nonselective opioid receptor antagonist naloxone partially attenuates the effects of \( \kappa \)-ORAs (Sengupta et al. 1996). In the present study, naloxone (2 mg/kg, given 10–15 min before a U50,488 stereoisomer) partially attenuated the effect of 8 mg/kg (1S, 2S)-U50,488, from 56.9 ± 4.9\% of control to 69.1 ± 3.4\% of control (\( n = 5; P < 0.05 \)). However, naloxone did not block the effect of 8 mg/kg (1R, 2R)-U50,488 (from 47.9 ± 9.2\% of control to 53.4 ± 9.7\% of control; \( n = 6; P > 0.05 \)).

**Effects of \( \kappa \)-ORAs dynorphin (1–13) and ICI 204,488**

The effects of cumulative doses (16 mg/kg) of dynorphin (1–13) or ICI 204,488 were tested on the responses to noxious CRD (80 mmHg, 30 s) of 16 mechanosensitive afferent fibers [dynorphin (1–13): \( n = 7 \); ICI 204,488: \( n = 9 \)]. Neither \( \kappa \)-ORA affected responses to CRD; the data are summarized in Fig. 3B.
However, both κ-ORAs dose-dependently decreased heart rate (Fig. 4).

**Effects of sodium channel blockers**

Mexiletine and carbamazepine, sodium channel blockers, dose-dependently attenuated responses of mechanosensitive afferent fibers to noxious CRD (cumulative dose, 16 mg/kg; the data are summarized in Fig. 3C). The mean ID_{50} of mexiletine (9.1) was not different from stereoisomers of U50,488 (see Table 1), but the mean ID_{50} of carbamazepine (19.2) differed from other drugs. The slopes of the dose-response functions of all drugs tested, however, were similar, which supports that the inhibitory action of the drugs on pelvic nerve afferent fibers occurs by a similar mechanism (Table 1). Both drugs dose-dependently reduced heart rate (P < 0.05; summarized in Fig. 4).

The effect of a mixture of one-half the ID_{50} doses of U50,488 and mexiletine was tested on responses of five mechanosensitive afferent fibers to noxious CRD (80 mmHg, 30 s). The inhibition of responses by U50,488 (ID_{50} dose), mexiletine (ID_{50} dose), and a mixture of U50,488 and mexiletine (½ ID_{50} doses of each) was comparable; the drug treatments decreased pelvic nerve afferent fiber responses to CRD to 56.4 ± 6.8%, 49.2 ± 3.4%, and 44.6 ± 6.5% of control, respectively, again supporting a common mechanism of action.

**Sodium channel recording**

Voltage-activated sodium currents were isolated by stepping from a holding potential of −80 mV to test potentials from −50 to +45 mV. The maximum current amplitude at 0 mV averaged −6 ± 0.45 nA (range −8.75 to −1.41 nA).

**U50,488 and stereoisomers inhibit voltage-activated sodium currents**

Figure 5A shows inhibition of the sodium current after perfusion of (1S,2S)-U50,488 (10^{-4} M); the inhibition was reversible on washing. In this experiment, voltage steps of 40 ms duration were applied every 20 s from a resting potential of −80 mV to a test potential of −30 mV. Figure 5B shows an example from a single cell that was exposed to (1S,2S)-U50,488 (10^{-5} M). In this experiment, voltage steps of 40 ms duration were applied from a resting potential of −80 mV to test potentials from −55 mV to +45 mV. (1S,2S)-U50,488 significantly inhibited voltage-activated sodium currents (P < 0.05). Current-voltage curves before and after (1S,2S)-U50,488 from three colon sensory neurons are summarized in Fig. 5C.

Similarly, the stereoisomers of U50,488, (1R,2R)-U50,488, and (1S,2R)-U50,488, also attenuated voltage-activated sodium currents. Figure 5D illustrates that peak sodium currents were inhibited by all stereoisomers of U50,488 (Student’s t-test; P < 0.05).

**U50,488-mediated sodium current reduction is tonic and use-dependent**

Voltage-activated sodium currents were evoked when voltage steps of 40 ms duration were applied from a resting
The addition of (1S,2S)-U50,488 (33.0 ± 5.5) when the cells were dialyzed with GDP-H9252 inhibited voltage-activated sodium currents to 66.1 ± 2.3% control (P < 0.05). The inhibition of sodium currents by (1S,2S)-U50,488 was further decreased to 80.2 ± 6.6% (P < 0.05). Because this decrease was less than that tested immediately after drug application, tonic inhibition is not time-dependent. The inhibition of sodium currents by (1S,2S)-U50,488 was also use-dependent as demonstrated by a progressive decrease in current during repetitive stimulation at 0.5 Hz. After 10 μM (1S,2S)-U50,488, the ratio of current amplitude at the 15th pulse decreased to 48.3 ± 5.9% (n = 3, P < 0.05). In control experiments, a second stimulus pulse was given after 30 s without repetitive depolarization; peak Na+ current decreased to 80.2 ± 6.6% (P < 0.05). This decrease was less than that tested immediately after drug application, tonic inhibition is not time-dependent. The inhibition of sodium currents by (1S,2S)-U50,488 was further decreased to 43.4 ± 7.0% after repetitive stimulation.

Nonopioid mediated effects

The specificity of opioid effect is determined by sensitivity to blockade by opioid receptor antagonists and by testing for the involvement of G proteins. Figure 7A shows the effect of application of naloxone (10^-4 M) at a concentration that is opioid receptor nonselective. Although naloxone alone inhibited voltage-activated sodium currents, it did not block the inhibitory effects of (1S,2S)-U50,488 (39.8 ± 8.1% of control; n = 4; P < 0.05). The peak sodium current also decreased after the addition of (1S,2S)-U50,488 (33.0 ± 7.5% of control; n = 5) when the cells were dialyzed with GDP-β-S, ruling out a G protein–mediated effect (P < 0.05; Fig. 7B).

DISCUSSION

We report here that the arylobenzacetamide κ-ORAs U50,488 and its enantiomers attenuate behavioral visceromotor responses to noxious CRD in awake rats with a rank order of potency of (1S,2S)-U50,488 > U50,488 >> (1R,2R)-U50,488. In complementary electrophysiological studies, U50,488 stereoisomers inhibited responses of decentralized pelvic nerve afferent fibers to CRD in a dose-dependent manner with similar mean inhibitory doses (ID50s). Mexiteline and carbamazepine, two sodium channel blockers, mimicked the inhibition of pelvic nerve afferent fibers produced by U50,488 compounds, whereas the κ-ORAs dynorphin (1–13) and structurally-modified arylobenzacetamide ICI 204,488 were ineffective. In preliminary studies, U50,488 and its stereoisomers were found to tonically and use-dependently inhibit voltage-activated sodium currents in colon sensory neurons.

Stereospecificity of opioid action

Typically, opioid receptors show a striking degree of stereospecificity for different isomers of their specific ligands, and it is well-known that enantiomers often produce different and sometimes opposite effects. Optically pure enantiomers of U50,488 have been synthesized (De Costa et al. 1987). Two enantiomers of trans-U50,488, (1S,2S)-U50,488 and (1R,2R)-U50,488, bind to κ-opioid receptor sites labeled by [3H]U69,593 with a 336-fold degree of enantioselective preference for the (1S,2S)-U50,488 enantiomer (Rothman et al. 1988). Alterations in the stereochemistry of U50,488 (trans- to cis-orientation) produces ligands relatively selective for opioid receptor sites labeled by [3H]U69,593.

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currents produced by U50,488 stereoisomers (10^{-4} M) and the inhibition was reversible on washing. Voltage steps of 40 ms duration were applied every 20 s from a resting potential of −80 mV to a test potential of −30 mV. B: example of data from a single cell that was exposed to (1S,2S)-U50,488 (10^{-5} M), which inhibited voltage-gated Na^+ currents. C: current-voltage curves before and after (1S,2S)-U50,488 (drug effect was significant; ANOVA; P < 0.05). D: inhibition of peak sodium currents produced by U50,488 stereoisomers (10^{-3} M; Student’s t-test; P < 0.05).

FIG. 5. Voltage-activated sodium currents were inhibited by stereoisomers of U50,488. A: sodium currents were inhibited after perfusion of (1S,2S)-U50,488 (10^{-4} M) and the inhibition was reversible on washing. Voltage steps of 40 ms duration were applied every 20 s from a resting potential of −80 mV to a test potential of −30 mV. B: example of data from a single cell that was exposed to (1S,2S)-U50,488 (10^{-5} M), which inhibited voltage-gated Na^+ currents. C: current-voltage curves before and after (1S,2S)-U50,488 (drug effect was significant; ANOVA; P < 0.05). D: inhibition of peak sodium currents produced by U50,488 stereoisomers (10^{-3} M; Student’s t-test; P < 0.05).

FIG. 6. (1S,2S)-U50,488–mediated reduction of sodium currents is tonic and use-dependent. Repeated tests of 40 ms duration were applied from a resting potential of −80 mV to a test potential of +10 mV at 0.5-Hz frequency. A: raw traces of currents of a cell tested with (1S,2S)-U50,488 (10^{-5} M) added to the bath solution. Sodium currents (as % control) from 3 cells as a function of time after (1S,2S)-U50,488 is shown below. B: repeated test potentials were given 30 s after (1S,2S)-U50,488 (10^{-5} M) was added to the bath solution. Sodium currents (as % control) from 3 cells as a function of time are shown below.

Nonopioid, sodium channel blocking actions of κ-ORAs

Several agonists acting at the κ opioid-receptor possessing an arylbenzacetamide structure have also been documented to inhibit voltage-activated sodium channels in cardiac myocytes (Pugsley et al. 1993, 1994) and hippocampal CA3 neurons (Alzheimer and Bruggencate 1990) by a nonopioid mechanism. The effect of these compounds on sodium channels on primary sensory neurons has not as yet been reported.

κ-ORAs such as U50,488 and CI 977 have been shown to decrease blood pressure and heart rate, and reduce the incidence and severity of ischemic and electrical arrhythmias in rats; these effects persisted in the presence of κ-opioid receptor blockade (Pugsley et al. 1992a,b). Subsequent electrophysiological studies have demonstrated that this nonopioid-dependent effect of U50,488 and its stereoisomers could be due to a blockade of voltage-activated sodium channels in cardiac myocytes (Pugsley et al. 1993, 1994), a mechanistic observation also replicated in hippocampal CA3 neurons (Alzheimer and Bruggencate 1990). In the present studies, we found that all U50,488 stereoisomers dose-dependently decreased heart rate in a nonenantioselective manner. Although producing no effect on pelvic nerve responses to CRD, dynorphin (1–13) and ICI 204,488 also decreased heart rate. The present experiments were not designed to determine whether effects on heart rate are central and/or peripheral or on cardiac sodium channels.

Peripheral, visceral site of action of U50,488 compounds

κ-ORAs have previously been documented to exert visceral antinociceptive effects by acting at peripheral and supraspinal sites. κ-ORAs, for example, when administered systemically or intracerebroventricularly, attenuate responses to noxious CRD and increase the visceromotor threshold for response (Burton and Gebhart 1998; Danzebrink et al. 1995; Harada et al. 1995a,b); intrathecal administration of the κ-OPA U50,488 is without effect. Consistent with these results, subcutaneous administration of U50,488 compounds in the present experiments attenuated the behavioral VMR to noxious CRD in (Bansinath et al. 1991), inhibition of gastrointestinal transit (Ramabadran et al. 1988), and anti-inflammatory/anti-arthritic effects (Wilson et al. 1996).
unanesthetized, awake rats in an enantiomer-selective manner. (1S,2S)-U50,488, the enantiomer with the greatest potency toward the \( \kappa \)-opioid receptor (Rothman et al. 1989; Zhu and Im 1992), was twice as effective as the standard analgesic \( \kappa \)-ORA, U50,488, while (1R,2R)-U50,488, the enantiomer that is without activity at the \( \kappa \)-opioid receptor, was the least potent in the present studies, but still effective at high doses. The \( \kappa \)-opioid receptor antagonist nor-BNI significantly antagonized the effects of only the (1S,2S)-U50,488 enantiomer. The analgesic effects of 10 mg/kg (±)-U50,488 could not be significantly blocked by nor-BNI, which is consistent with previous reports (Burton and Gebhart 1998). These results support the suggestion that a nonopioid mechanism contributes to the visceral antinociception produced by systemically administered U50,488 compounds.

The present single fiber electrophysiology results confirm the findings of previous studies (Sengupta et al. 1996, 2000; Su et al. 1997a,b) that documented that \( \kappa \)-ORAs (U50,488, U69,593, U62,066, EMD 61,753, bremazocine, nalBzoH, and fedotozine) can act at peripheral sites to dose-dependently inhibit responses of mechanosensitive pelvic nerve afferent fibers to noxious CRD in the rat. None of the putative \( \kappa_1 \), \( \kappa_2 \), or \( \kappa_3 \) opioid receptors characterized from binding studies were sites at which antinociception was produced in these previous studies (Su et al. 1997b). We also noted that naltrexone could only partially antagonize the effects of the \( \kappa \)-ORAs tested (Sengupta et al. 1996; Su et al. 1997a). Further, the effects also could not be blocked by nor-BNI or DIPPA, which are reported to be selective \( \kappa \)-opioid receptor antagonists (Sengupta et al. 1996; Su et al. 1997b). We subsequently employed an antisense oligodeoxynucleotide to specifically knock down the cloned rat \( \kappa \)-opioid receptor at peripheral, visceral sites; the \( \kappa \)-ORA EMD 61,753 was still able to dose-dependently attenuate responses of pelvic nerve afferent fibers to noxious CRD in these experiments (Joshi et al. 2000). Because these \( \kappa \)-ORAs tested in pelvic nerve afferent fiber recordings were arylenzacetamide analogues, we tested in the present experiments the highly selective \( \kappa \)-ORA (1S,2S)-U50,488 and its less selective enantiomer (1R,2R)-U50,488 and a diastereomer (1S,2R)-U50,488. As discussed above, the pharmacology of these stereoisomers for the \( \kappa \)-opioid receptor is vastly different, and they also have nonopioid sodium channel blocking activity.

We also tested \( \kappa \)-ORAs with different chemical structures and no documented activity on sodium channels [dynorphin (1–13) and ICI 204,488]. Surprisingly, the peptide dynorphin (1–13) and a structurally-modified arylenzacetamide \( \kappa \)-ORA ICI 204,488 failed to attenuate pelvic nerve responses to CRD. In contrast, all U50,488 compounds dose-dependently inhibited the responses of pelvic nerve afferent fibers and did so with equal potency. In addition, two drugs with sodium channel blocking activity (mexiletine and carbamazepine) inhibited responses of pelvic nerve afferent fibers to CRD with similar mean inhibitory doses (ID\(_{50}\)) (Table 1). In the aggregate, these results support the suggestion that sodium channels are a common peripheral, non-\( \kappa \)-opioid receptor site of action for these agents.

**Mechanism of U50,488 induced suppression of pelvic nerve activity**

Modulation of pelvic nerve activity by U50,488 compounds could thus occur principally by blockade of sodium channels. We attempted to directly address this suggestion in the present study in two ways. First, we tested the effects of sodium channel blockers mexiletine and carbamazepine on response of pelvic nerve afferent fibers to CRD. Second, we tested the effects of (1S,2S)-U50,488, (1S,2R)-U50,488 and (1R,2R)-U50,488 on voltage-activated sodium currents in colon sensory neurons.

In humans, neuropathic pain can be relieved by use-dependent sodium channel blockers, carbamazepine and mexiletine (Tanelian and Brose 1991). Intravenous administration of mexiletine (3–15 mg/kg) or the local anesthetic lidocaine (5–25 mg/kg) have been shown to decrease the sensitivity of spontaneously active fibers in rat sciatic neuramors to mechanical stimulation (Chabal et al. 1989). Over a similar dose range, we found that mexiletine attenuated responses of mechanosensitive pelvic nerve afferent fibers to noxious CRD. The ID\(_{50}\) and slope of dose-response functions were similar with that of U50,488 compounds. Experiments combining half doses of ID\(_{50}\) of mexiletine with (1S,2S)-U50,488 did not produce effects different from their individual ID\(_{50}\) doses, supporting a similar mechanism of inhibitory action for these two classes of compounds.

In the whole cell patch-clamp experiments, perfusion of U50,488 compounds decreased voltage-activated sodium current in colon sensory neurons in a concentration-dependent manner.
manner. These effects were not opioid receptor-mediated because 1) the inhibition of sodium currents was not stereospecific, 2) a nonselective opioid receptor antagonist naloxone (10^-4 M) did not reverse the inhibitory effect of (1S,2S)-U50,488, and 3) the inhibition was not G protein-mediated. Similar nonopioid effects resulting from blockade of sodium channels by α-ORAs have been reported for U50,488 in hippocampal CA3 neurons (Alzheimer and Bruggencate 1990) and by the anticonvulsant α-ORA U54494A in neuroblastoma cells (Zhu and Im 1992). We have not yet determined whether there are preferential effects of these drugs on sodium currents sensitive or resistant to tetrodotoxin (TTX), but these and related experiments are currently under way.

The effective concentrations of U50,488 compounds are comparable to the local anesthetic lidocaine for inhibiting sodium channels in amphibian peripheral nerves (half-maximal inhibitory concentration, IC50, 172 μM) (Bräu et al. 2000) and recombinant voltage-dependent sodium channels (IC50, 1.9 mM) (Wagner et al. 1999).

Inhibition of sodium currents by U50,488 is tonic and use-dependent. Tonic inhibition arises from channel blockade in the absence of recent activity (e.g., resting and possibly open channels). Phasic or use-dependent inhibition is a progressive depression of current during repetitive stimulation arising from the accumulation of long-lasting, drug-blocked states. Thus U50,488 might directly act on the channel pore like local anesthetics. The ability of sodium channel blockers to be frequency-dependent in their action is essential for their clinical utility since they presumably only target spontaneously active nerves while not affecting conduction in normal nerves. Furthermore, sodium channels spend more time in the open and inactive states in pathological conditions (Catterall 1987; Tanelian and Brose 1991; Wagner et al. 1999).

**Significance**

Voltage-activated sodium channels are responsible for the initial rapid membrane depolarization during an action potential in neurons. Colon sensory neurons contain both TTX-sensitive and TTX-resistant sodium currents (Su et al. 1999). Sodium channels could have an exaggerated activity and/or could be up-regulated in inflammatory conditions. The use of agents known to block sodium channels in a use-dependent fashion (e.g., mexiletine, carbamazepine) and antidepressants are employed with some success in management of pain in humans (Tanelian and Brose 1991). In the present study, we provide evidence to support the suggestion that inhibition of voltage-activated sodium currents in colon sensory neurons by the β-ORA U50,488 and its stereoisomers could contribute to their visceral antinociceptive effects. Such blockade of sodium channels may represent another pharmacological mechanism for treatment of discomfort and pain associated with functional bowel disorders such as irritable bowel syndrome.

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