Nonopioid Actions of U50,488 Enantiomers Contribute to Their Peripheral Cutaneous Antinociceptive Effects

S. K. JOSHI and G. F. GEBHART

Department of Pharmacology, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, Iowa City, Iowa

Received January 15, 2003; accepted February 25, 2003

ABSTRACT

The ability of arylacetamide κ-opioid receptor agonists (κ-ORAs) to block sodium channels by a nonopioid mechanism has been previously documented. The present experiments were undertaken to test whether two enantiomers of the arylacetamide κ-ORA (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide (U50,488), (+)-(1*R*,2*R*)-U50,488 and (−)-(1*S*,2*S*)-U50,488, are antinociceptive in the formalin model by a peripheral, nonopioid receptor-mediated mechanism. Although both enantiomers have been previously shown to block sodium channels with comparable potencies, only (−)-(1*S*,2*S*)-U50,488 has activity at the κ-opioid receptor (KOR). In the formalin test, intrapaw administration of U50,488 enantiomers as well as lidocaine exhibited significant dose-related attenuation of formalin-induced flinching behavior. The rank order of potency of the drugs tested was (−)-(1*S*,2*S*)-U50,488 > (+)-(1*R*,2*R*)-U50,488 > lidocaine. The antinociception produced by lower doses of (−)-(1*S*,2*S*)-U50,488 was blocked by intrapaw nor-binaltorphimine as well as by antisense knockdown of the KOR. Such pretreatments, however, did not block the antinociception produced by (+)-(1*R*,2*R*) U50,488, lidocaine, or higher doses of (−)-(1*S*,2*S*)-U50,488. These data suggest that the sodium channel blocking effects of U50,488 and similar κ-ORAs can contribute to their peripheral antinociceptive actions.

Due to undesirable side effects (e.g., respiratory depression and constipation), development of analgesic tolerance, and abuse potential associated with morphine and other μ-opioid receptor agonists (ORAs), there has been a continuing interest in developing agonists at the other opioid receptors, including the κ-opioid receptor (KOR). However, the clinical utility of κ-ORAs has been compromised due to undesirable central nervous system side effects like dysphoria and psychotomimesis (Millan, 1990). One strategy to circumvent the central nervous system side effects associated with opioids has been to take advantage of analgesia that can be produced by opioids at peripheral sites (Stein et al., 2001). Accordingly, κ-ORAs have been shown to be antinociceptive when restricted to peripheral cutaneous sites such as the tail (Kolesnikov et al., 1996) and hind paw (Joshi et al., 2000) as well as peripheral visceral sites such as the colon (Su et al., 2000).

In addition to activity at the KOR, several κ-ORAs have been shown to have nonopioid pharmacological actions. Arylacetamide κ-ORAs share important structural similarities

with local anesthetics such as lidocaine (Pugsley et al., 2000) and have been documented in several studies to possess voltage-gated sodium channel blocking properties. A series of experiments have established that the cardiovascular effects of κ-ORAs such as antiarrhythmia and hypotension occur due to their sodium channel blocking actions in cardiac myocytes (Pugsley et al., 1993, 1994). Such actions of κ-ORAs have also been demonstrated in hippocampal CA3 neurons (Alzheimer and Bruggencate, 1990), neuroblastoma cells (Zhu and Im, 1992), primary sensory neurons in the dorsal root ganglia (Su et al., 2002), and in oocytes heterogeneously expressing sodium channels (Pugsley et al., 2000). The blockade of voltage-gated sodium channels in these studies was opioid-receptor independent, as verified by use of opioid receptor antagonists.

The synthesis of enantiomers of some traditionally used arylacetamide κ-ORAs has made available another strategy to examine whether a particular pharmacological action of a κ-ORA involves opioid or nonopioid receptor-mediated actions (e.g., voltage-gated sodium channel blockade). The optically pure enantiomers of the traditionally used κ-ORA analgesic (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidi-nyl)-cyclohexyl]benzeneacetamide (U50,488) [i.e., (±)-*trans*-

---

ABBREVIATIONS: ORA, opioid receptor agonist; KOR, κ-opioid receptor; nor-BNI, nor-binaltorphimine; ODN, oligodeoxynucleotide; U50,488, (*trans*)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide; U69,593, (5α,7α,8β)-(−)-*N*-methyl-*N*-(7-(1-pyrrolidinyl)-1-oxaspiro (4,5)dec-8-yl) benzeneacetamide; PD 129,289, [5*R*-(5α,7α,8β)]-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzofuranacetamide Hcl; PD 129,290, [5*S*-(5α,7α,8β)]-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-4-benzofuranacetamide Hcl; EMD 61,753, (*N*-methyl-*N*-[(1*S*)-1-phenyl)-2-(13*S*)-3-hydroxypyrrolidine-1-yl)-ethyl]-2,2-diphenylacetamide HCl.

U50,488] have been synthesized and their in vitro receptor selectivities and pharmacological activities described (Rothman et al., 1989; Pugsley et al., 1993). The (−)-trans-(1S,2S)-U50,488 enantiomer exhibits a greater affinity toward the KOR (K_d = 0.89 nM) and about 4-fold greater antinociceptive potency than U50,488. In contrast, the (+)-trans-(1R,2R)-U50,488 enantiomer has insignificant affinity toward the KOR (K_d = 299 nM) and is without effect in KOR-mediated antinociceptive assays. Although U50,488 and both its enantiomers each have a distinct opioid pharmacology, they display comparable potencies in producing a block of voltage-gated sodium channels by a nonopioid mechanism (Pugsley et al., 1993; Su et al., 2002).

We have previously documented the ability of arylacetamide κ-ORAs to produce antinociception at peripheral sites (Joshi et al., 2000). However, it is not known whether κ-ORAs could produce such antinociception through nonopioid mechanisms, such as sodium channel blockade. Accordingly, in the present study, we used the two U50,488 enantiomers to test the hypothesis that arylacetamide κ-ORAs are antinociceptive at a peripheral site by a nonopioid mechanism. We examined the antinociceptive actions of peripherally administered (+)-(1R,2R)-U50,488 and (−)-(1S,2S)-U50,488 in the formalin test. The ability of the KOR antagonist nor-BNI and KOR antisense oligodeoxynucleotides to block these antinociceptive actions was also examined. Because the nonopioid antinociceptive actions of the U50,488 enantiomers could be due to their sodium channel blocking properties, we verified that peripherally administered lidocaine also produced antinociception in the formalin test, and compared its antinociceptive potency to that of the U50,488 enantiomers.

Materials and Methods

Animals. Male Sprague-Dawley rats (Harlan, Indianapolis, IN), housed one to two per cage with free access to food and water, were maintained on a 12-h light/dark cycle (lights on 6:00 AM–6:00 PM) in the Association for the Assessment and Accreditation of Laboratory Animal Care-approved animal care facility. The Institutional Animal Care and Use Committee of The University of Iowa approved all experimental procedures.

Behavioral Study. The antinociceptive effects of peripherally administered drugs were studied by evaluating their ability to attenuate the flinching response after injection of formalin into the dorsal surface of a rat hind paw. Flinching of the injected paw is a consistent component of formalin-induced behavior and has been advocated as a more robust parameter, compared with paw licking, and less contaminated by other non-nociceptive behavioral changes (Tjolsen et al., 1992). Different groups of rats (n = 4–7) received different doses of lidocaine, (+)-(1S,2S)-U50,488, (−)-(1R,2R)-U50,488, or vehicle, injected into the dorsum of the right hind paw, 10 min before formalin injection (50 μl, 2.5%) at the same site. The volume of drug injections ranged from 25 to 75 μl. Rats were then placed in observation chambers and paw flinches were counted in bins of 5 min, starting with the formalin injection and continuing for 50 min. The flinch response was divided into first and second phases by summing the total number of flinches occurring between 0 and 15 and between 15 and 50 min, respectively. The antinociception produced by drugs in the presence of KOR blockade brought about either by intrapaw pretreatment (1 min) with the KOR antagonist nor-BNI (50 μg), or intrathecal pretreatment with KOR antisense oligodeoxynucleotides was also evaluated. Animals were tested with vehicle (saline or 10% ethanol) each time an experiment with a drug treatment group was performed, and data for vehicle-treated rats presented in figures reflects results pooled from all experiments. In preliminary experiments, the highest doses of the drugs tested here were not found to attenuate the formalin-induced flinching behavior when administered into the contralateral hind paw and the tail vein, thus verifying the peripheral effect of lidocaine and the U50,488 enantiomers upon their intrapaw administration.

Synthesis and Administration of Oligodeoxynucleotides (ODNs). The ability of KOR antisense ODNs to block the antinociceptive effect of lidocaine, (+)-(1R,2R)-U50,488 and (−)-(1S,2S)-U50,488 was evaluated in separate groups of animals. Intrathecal (i.t.) administration of antisense, but not mismatch ODN has been previously shown by us to cause a peripheral down-regulation of the KOR and block peripheral κ-ORA-mediated antinociception in the formalin test, without affecting antinociception mediated at other opioid receptors (Joshi et al., 2000).

The synthesis and administration of the ODNs have been described previously (Joshi et al., 2000) and are only briefly summarized here. Antisense and mismatch ODNs had a phosphodiester backbone, derived from the 5′ end of the coding sequence of the cloned rat KOR (nucleotides 6–25) and were synthesized by Integrated DNA Technologies (Iowa City, IA) and reconstituted in sterile deionized water before use. The sequences of the two ODNs used in these experiments were as follows: 5′-GGAAAATCTG GATGGGG-GAC-3′ (antisense) and 5′-GGAAATACTG GTAGGGGAGC-3′ (mismatch).

Rats were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg, Nembutal; Abbott Laboratories, Abbott Park, IL) and a catheter (8.5 cm; polyethylene-10) was passed to the lumbosacral i.t. space through an incision in the dura over the atlantooccipital joint. Rats were allowed at least 3 days to recover from surgery before testing. Antisense or mismatch ODNs were injected i.t. twice daily (dosing interval 10–12 h) for four consecutive days, each dose of ODN containing 12.5 μg in a 5-μl volume followed by a 10-μl saline flush. The formalin test was carried out on the morning of the 5th day, approximately 12 h after the last bolus ODN dose, and the antinociception produced by lidocaine, (+)-(1R,2R)-U50,488 and (−)-(1S,2S)-U50,488 was evaluated in the ODN-treated rats.

Drugs. (+)-(1R,2R)-U50,488 (mol. wt. 521.5; Sigma-Aldrich, St. Louis, MO) was dissolved in 10% ethanol. (−)-(1S,2S)-U50,488 (mol. wt. 521.5; Sigma-Aldrich) was dissolved in normal saline, or 10% ethanol for higher concentrations. Lidocaine hydrochloride (4%) was obtained from Roxane Laboratories, Inc. (Columbus, OH). Nor-BNI dihydrochloride (mol. wt. 734.7; Sigma/RBI, Natick, MA) was dissolved in normal saline. The lack of effects of saline and ethanol were determined in preliminary experiments.

Data Analysis. Experimental groups consisted of four to seven rats, and data are presented as mean ± S.E.M. The data were analyzed by one-way analysis of variance with Dunnett's test for post hoc comparisons of different treatment groups with vehicle control. A value of $P < 0.05$ was considered statistically significant in all tests. Effective dose 50 (ED_{50}; dose reducing the second phase flinching response by 50%) and 95% confidence intervals were calculated from the 20 to 80% component of the dose-response curve (Tallarida and Murray, 1991).

Results

Effect of Lidocaine and (+)-(1R,2R)-U50,488 in the Formalin Test. Injection of formalin (2.5%, 50 μl) into the dorsal surface of the hind paw produced characteristic biphasic flinching behavior with clear first (0–15-min) and second (15–50-min) phases (Fig. 1A). Drugs or vehicle were injected 10 min before formalin at the same site to evaluate their peripheral antinociceptive effects. Rats treated with vehicle flinched a mean 96 ± 7 (first phase) and 371 ± 23 (second phase) after formalin administration. Significant dose-related attenuation of the number of first and second phase
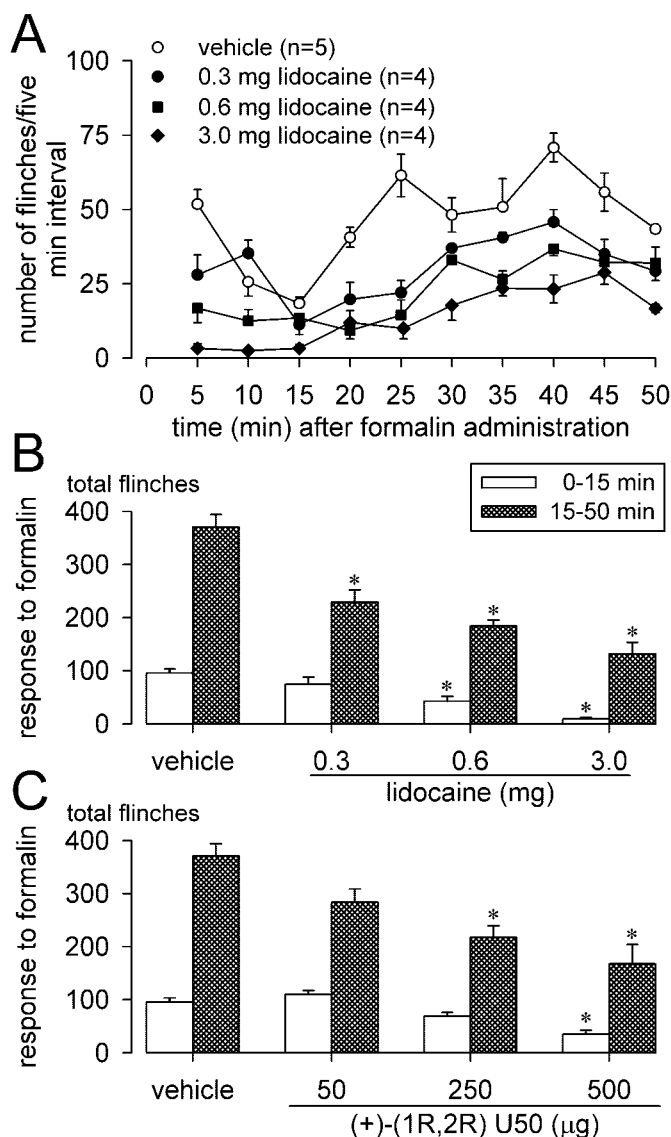
KOR antisense ODNs (12.5 μg in 5 μl) were administered i.t. twice daily for four consecutive days before examining drug effects in the formalin test. We have previously established that this pretreatment regimen causes a down-regulation of KOR in lumbosacral dorsal root ganglia and blocks peripheral κ-ORA-mediated antinociception in the formalin test (Joshi et al., 2000). Neither nor-BNI nor KOR antisense ODN pretreatment affected the antinociception produced by 3 mg of lidocaine (Fig. 2A) or 500 μg (+)-(1R,2R)-U50,488 (Fig. 2B), thus demonstrating an absence of KOR involvement in the antinociception produced by these drugs. Administration of KOR antisense or mismatch ODNs alone do not affect formalin-induced flinching behavior (Fig. 2C).
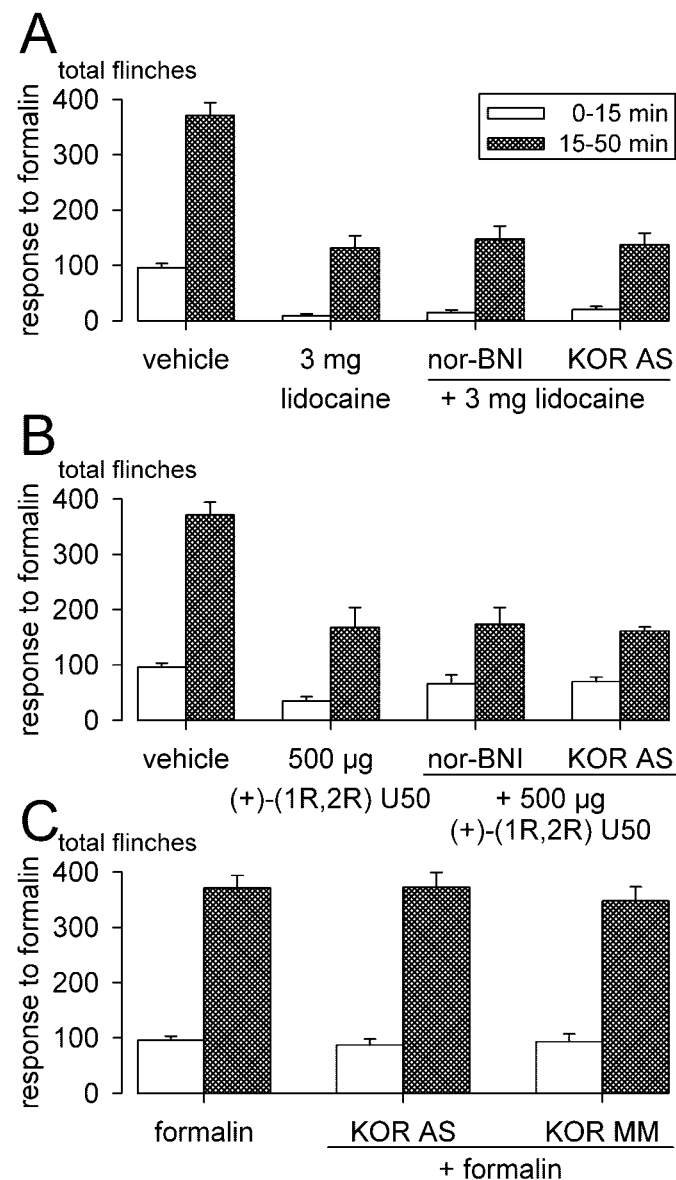

Fig. 1. Effect of lidocaine and (+)-(1R,2R)-U50,488 in the formalin test. Formalin (2.5%, 50 μl) was injected into the dorsal surface of the rat hind paw 10 min after injection of vehicle or drugs at the same site. A, time course of formalin-induced flinching behavior for groups of animals pretreated with either vehicle or different doses of lidocaine exhibits a characteristic biphasic response. Lidocaine dose dependently attenuated the formalin-induced flinching response. Data are summarized in B. The total number of flinches in the first and second phases of the formalin test was dose dependently attenuated by pretreatment with lidocaine (B) and (+)-(1R,2R)-U50,488 (C) (*, $P < 0.05$, significantly different from corresponding vehicle-treated group). Data for each different treatment group were obtained from four to five animals.

flinches was observed after pretreatment with the sodium channel blocker lidocaine (Fig. 1B) and (+)-(1R,2R)-U50,488 (Fig. 1C).

Effect of KOR Blockade on Antinociception Produced by Lidocaine and (+)-(1R,2R)-U50,488. To examine the involvement of the KOR in antinociception produced by lidocaine and (+)-(1R,2R)-U50,488, we evaluated the antinociception produced by these drugs in the presence of KOR blockade brought about by two different approaches. In two groups of rats, the KOR antagonist nor-BNI (50 μg) was injected 1 min before the injection of either lidocaine or (+)-(1R,2R)-U50,488 at the same site. In other groups of rats,

Fig. 2. Effect of KOR blockade on antinociception produced by lidocaine and (+)-(1R,2R)-U50,488 in the formalin test. The antinociception produced by 3 mg of lidocaine (A) and 500 μg of (+)-U50,488 (B) was unaffected by pretreatment with either the KOR antagonist nor-BNI (50 μg, injected 1 min before lidocaine at the same site) or KOR antisense ODN (delivered i.t. into the lumbosacral space, twice daily for four consecutive days, each dose containing 12.5 μg of ODN in 5 μl). C, effect of KOR antisense (AS) or KOR mismatch (MM) ODN on formalin-induced flinching. Data for each different treatment group were obtained from four to five animals.

Effect of (−)-(1S,2S)-U50,488 in the Formalin Test. Similar to effects produced by lidocaine and (+)-(1R,2R)-U50,488, (−)-(1S,2S)-U50,488 produced significant dose-related attenuation of the number of first- and second-phase flinches in the formalin test (Fig. 3A). The (−)-(1S,2S)-U50,488 enantiomer, which has activities both as a κ-ORA and sodium channel blocker, was the most potent among the three drugs tested (summarized in Fig. 5).

Effect of KOR Blockade on Antinociception Produced by (−)-(1S,2S)-U50,488. The antinociceptive effect of the low 5-μg dose of (−)-(1S,2S)-U50,488 was prevented by pretreatment with either the KOR antagonist nor-BNI or KOR antisense ODNs (Fig. 3B). These results are consistent with previous reports documenting potent KOR agonist activity of the (−)-(1S,2S)-U50,488 enantiomer (Ramabadran et al., 1988; Rothman et al., 1989; Taub et al., 1991; Wilson et al., 1996). The antinociception produced by a 10-fold greater 50-μg dose of (−)-(1S,2S)-U50,488 was also significantly attenuated by KOR blockade (Fig. 4A). In contrast, the antinociception produced by 500 μg of (−)-(1S,2S)-U50,488, the dose at which the non-κ-ORA sodium channel blocker (+)-(1R,2R)-U50,488 also produces antinociception (Fig. 1C), was unaffected by KOR blockade (Fig. 4B). These results support an interpretation that (−)-(1S,2S)-U50,488 at low doses produces antinociception mediated by the KOR, but at higher doses can produce antinociception also by a mechanism independent of its actions at the KOR.
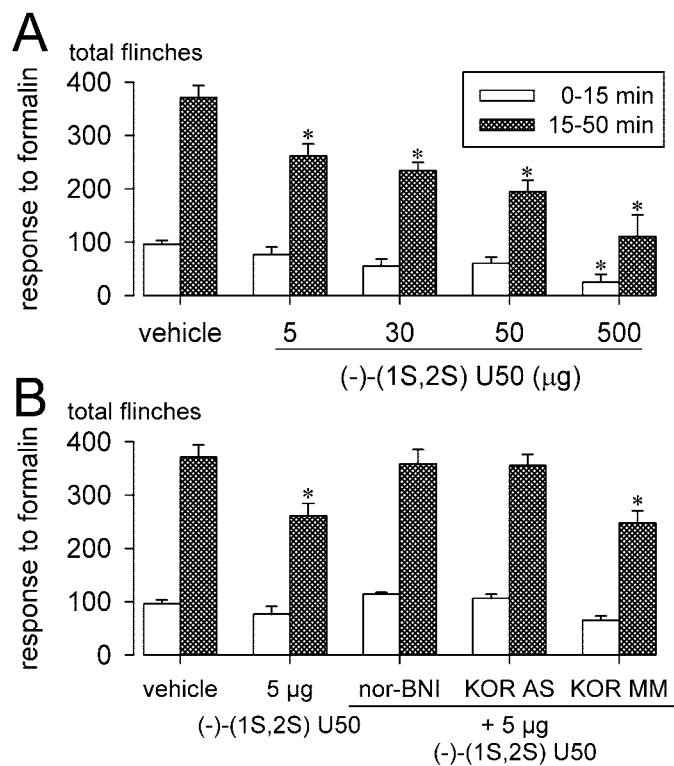
Fig. 4. Effect of KOR blockade on antinociception produced by 50 and 500 μg of (−)-(1S,2S)-U50,488 in the formalin test. Both nor-BNI and KOR antisense ODN significantly attenuated the antinociception produced by 50 μg of (−)-(1S,2S)-U50,488 (A; *, $P \leq 0.05$, significantly different from corresponding 50 μg of (−)-(1S,2S)-U50,488-treated group), but did not affect the antinociception produced by 500 μg of (−)-(1S,2S)-U50,488 (B). Data for each different treatment group were obtained from four to five animals.

Antinociceptive Potencies of Lidocaine, (+)-(1R,2R)-U50,488 and (−)-(1S,2S) U50,488. Antinociceptive dose-response curves for lidocaine, (+)-(1R,2R)-U50,488, and (−)-(1S,2S)-U50,488 were generated by plotting the effect of drug dose on the number of second phase flinches as percentage of antagonism relative to the number of second-phase flinches in vehicle-treated rats, which was taken as 100% (Fig. 5). As expected, (−)-(1S,2S)-U50,488 was the most potent, given its actions as a κ-ORA as well as a sodium channel blocker. The (−)-(1S,2S)-U50,488 dose-response curve in the presence of KOR blockade, produced by either nor-BNI or KOR antisense ODN, was shifted to the right, and was similar to the dose-response curve of the non-κ-ORA (+)-(1R,2R)-U50,488 enantiomer. Doses producing 50% antagonism of the second phase flinching response to formalin are given in Table 1. These data suggest that (−)-(1S,2S)-U50,488 can produce dose-dependent antinociception due to its sodium channel blocking actions, even upon blockade of its actions as a κ-ORA.

Fig. 3. Effect of (−)-(1S,2S) U50,488 in the formalin test. A, pretreatment with (−)-(1S,2S)-U50,488, administered into the dorsal surface of the rat hind paw 10 min before formalin, dose dependently attenuated the total number of flinches in the first and second phases of the formalin test (*, $P \leq 0.05$, significantly different from corresponding vehicle-treated group). B, antinociception produced by 5 μg of (−)-(1S,2S)-U50,488 was absent after either nor-BNI or KOR antisense (KOR AS), but not mismatch (KOR MM), ODN. Data for each different treatment group were obtained from four to seven animals.
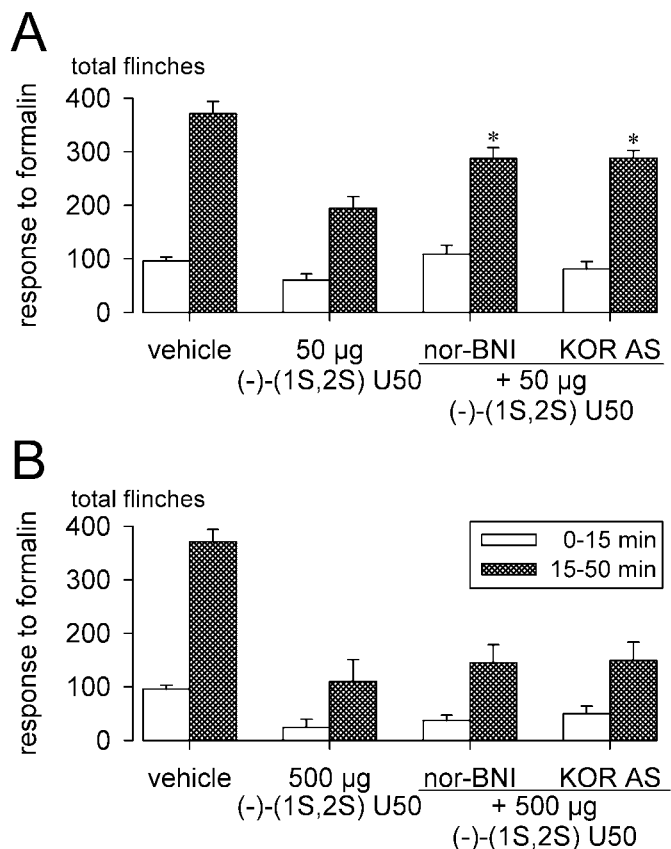
Discussion

The present results establish antinociceptive effects produced by peripherally administered sodium channel blockers and κ-ORAs, with a rank order of potency of (−)-(1S,2S)-U50,488 > (+)-(1R,2R)-U50,488 > lidocaine. The antinoci-
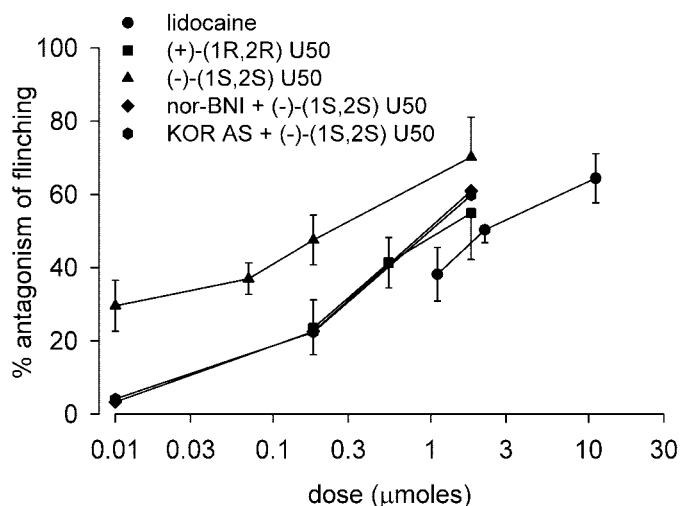
Fig. 5. Dose-response curves for antinociception produced by lidocaine, (+)-(1R,2R) U50,488, (−)-(1S,2S)-U50,488, and (−)-(1S,2S)-U50,488 in the presence of KOR blockade in phase two of the formalin test. Effects of drug are expressed as percentage of antagonism relative to the number of flinches in vehicle-treated rats (taken as 100%).

TABLE 1

ED_{50} values and 95% confidence intervals (95% CI), in micromoles, of dose-dependent drug effects in attenuating the second-phase flinching response to formalin

Drugs	ED_{50} (95% CI)
Lidocaine	2.81 (0.29–27.5)
(+)-(1R,2R)-U50,488	1.17 (0.23–6.02)
(−)-(1S,2S)-U50,488	0.2 (0.055–0.73)

ception produced by sodium channel blockers lidocaine and (+)-(1R,2R)-U50,488 was not affected by KOR blockade produced either by pretreatment with intrapaw nor-BNI or i.t. KOR antisense ODNs. In contrast, the (−)-(1S,2S)-U50,488 enantiomer, which has been previously documented to have activity both as a κ-ORA and sodium channel blocker, produced antinociception that at lower doses was attenuated by KOR blockade. The antinociception produced by the greatest dose of (−)-(1S,2S)-U50,488 tested, a dose at which the non-κ-ORA sodium channel blocker (+)-(1R,2R)-U50,488 also produced antinociception, was unaffected by KOR blockade. Pretreatment with either nor-BNI or KOR antisense ODNs shifted the (−)-(1S,2S)-U50,488 dose-response curve to the right, and the dose-response curve then was similar to that of the (+)-(1R,2R)-U50,488 enantiomer. These data support the hypothesis that the sodium channel blocking actions of U50,488 and similar arylacetamide κ-ORAs can contribute to their peripheral and central antinociceptive actions.

Nonopioid Actions of Arylacetamide κ-ORAs. Based on findings over the last decade, it is well appreciated that κ-ORAs have pharmacological effects independent of their actions at κ-opioid receptors. Arylacetamide κ-ORAs, including U50,488, like lidocaine, satisfy all structural features that have been suggested as necessary for sodium channel blockade (Pugsley et al., 2000) and blockade of voltage-gated sodium channels by κ-ORAs having an arylacetamide structure has been documented, mostly in the cardiovascular system. Early studies showed the ability of U50,488 and related κ-ORAs 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

KOR antagonists (Pugsley et al., 1992a,b). Subsequent electrophysiological studies confirmed that this nonopioid effect of U50,488 was due to a direct blockade of voltage-gated sodium channels in cardiac myocytes (Pugsley et al., 1993, 1994). The sodium channel blocking actions of κ-ORAs have been replicated in hippocampal CA3 neurons (Alzheimer and Bruggencate, 1990), neuroblastoma cells (Zhu and Im, 1992) and more recently in primary sensory neurons in the dorsal root ganglion (Su et al., 2002). Another line of evidence for opioid receptor-independent blockade of sodium channels by κ-ORAs such as U50,488 derives from experiments using heterogeneous expression of sodium channels in oocytes (Pugsley et al., 2000). In oocytes expressing only the rat heart voltage-gated sodium channel ($Na_V1.5$), and not the KOR, U50,488 produced a dose-dependent inhibition of peak sodium current, with potency greater than that of lidocaine.

Use of Enantiomers to Investigate Mechanism of Arylacetamide κ-ORA Action. Opioid receptors typically exhibit a striking degree of stereospecificity for different isomers of their specific ligands. After the synthesis of optically pure enantiomers of the traditionally used arylacetamide κ-ORA analgesic U50,488 [i.e., (±)-trans-U50,488], in vitro receptor selectivities and pharmacological activities of the two enantiomers were described (Rothman et al., 1989; Pugsley et al., 1993). In binding studies, the KOR exhibits a 336-fold degree of enantioselectivity for (−)-(1S,2S)-U50,488 over (+)-(1R,2R)-U50,488 (Rothman et al., 1989). Findings from KOR-related pharmacological activity studies are consistent with the results of these binding experiments. For example, the (−)-(1S,2S)-U50,488 enantiomer is 100-fold more potent than (+)-(1R,2R)-U50,488 in immunosuppression studies (Taub et al., 1991) and drug discrimination assays (Rothman et al., 1989). The stereospecificity of KOR effects has also been reported in antinociceptive assays (Rothman et al., 1989), induction of convulsions (Bansinath et al., 1991), inhibition of gastrointestinal transit (Ramabadran et al., 1988), and anti-inflammatory/antiarthritic effects (Wilson et al., 1996).

The structural similarity but distinct opioid pharmacology of enantiomers of arylacetamide κ-ORAs makes them a valuable tool to investigate whether a particular pharmacological action of the κ-ORA involves an opioid or nonopioid mechanism. For example, the antiarrhythmic efficacy of arylacetamide was maintained when enantiomeric pairs such as (+)-PD 129,290 (which lacks affinity for the KOR) and (−)-PD 129,289 (which has high affinity for the KOR) were examined (Pugsley et al., 1993), giving further support to the notion that these cardiac effects were not mediated by the KOR, but by sodium channel blockade. In studies of visceral nociception, systemically administered (±)-trans-U50,488, (+)-(1R,2R)-U50,488, and (−)-(1S,2S)-U50,488 produced dose-dependent antinociception and also dose dependently attenuated responses of decentralized pelvic nerve afferent fibers to noxious colon distension, the latter by a KOR-independent mechanism, suggesting contribution of sodium channel blockade by a nonopioid mechanism (Su et al., 2002). This hypothesis was directly addressed by investigating the effects of (−)-(1S,2S)-U50,488 and (+)-(1R,2R)-U50,488 on voltage-gated sodium currents in colon sensory neurons in the S1 dorsal root ganglion. Both U50,488 enantiomers decreased voltage-gated sodium currents with comparable potencies even in the presence of opioid receptor antagonism

(Su et al., 2002). Such findings demonstrate that the two U50,488 enantiomers have comparable potency in blocking voltage-gated sodium channels, but drastically different actions on the KOR.

Peripheral Antinociception Produced by Sodium Channel Blockade. We and others have previously documented the ability of arylacetamide κ-ORAs to produce antinociception at peripheral sites (Kolesnikov et al., 1996; Joshi et al., 2000; Su et al., 2000). In one such study, intrapaw administration of arylacetamide κ-ORAs U69,593 and EMD 61,753 attenuated the flinching response to injection of subcutaneous formalin into the rat hind paw (Joshi et al., 2000). Because arylacetamide κ-ORAs have been shown to inhibit voltage-gated sodium currents in primary sensory neurons (Su et al., 2002), the peripheral antinociception produced by these drugs could also be a result of such mechanism. In the present study, we addressed this issue by using the two enantiomers of U50,488 [i.e., the potent κ-ORA (−)-(1S,2S)-U50,488 and the non-κ-ORA (+)-(1R,2R)-U50,488]. As discussed above, the two enantiomers have comparable potencies in mediating sodium channel blockade. We report here that intrapaw administration of sodium channel blockers such as lidocaine and (+)-(1R,2R)-U50,488 produce dose-related antinociception in the formalin test. Not surprisingly, the (−)-(1S,2S)-U50,488 enantiomer, which has activity both as an opioid receptor agonist and sodium channel blocker, was the most potent of the three drugs tested in the present experiments. To test whether the sodium channel blocking action alone of (−)-(1S,2S)-U50,488 could produce peripheral antinociception in the formalin test, we used two approaches to block the effects of (−)-(1S,2S)-U50,488 at the KOR at peripheral sites. First, we injected 50 μg of nor-BNI in the hind paw before and at the same site as (−)-(1S,2S)-U50,488 and formalin injection and saw a blockade of the antinociceptive actions of lower doses of (−)-(1S,2S)-U50,488. The second approach used was pretreatment with antisense ODNs targeting the KOR. Consistent with previous findings (Joshi et al., 2000) and the present observations with nor-BNI, KOR antisense ODN (but not mismatch ODN) attenuated the antinociception produced by lower doses of (−)-(1S,2S)-U50,488. As expected, neither strategy to block the KOR affected the antinociception produced by lidocaine or (+)-(1R,2R)-U50,488. Interestingly, when the dose of (−)-(1S,2S)-U50,488 was increased to the effective doses of the (+)-(1R,2R)-U50,488 enantiomer, the resulting antinociception was also resistant to KOR blockade. Pretreatment with either nor-BNI or KOR antisense ODN shifted the (−)-(1S,2S)-U50,488 dose-response curve to the right, and the dose-response curve then resembled that of the (+)-(1R,2R)-U50,488. These findings support the notion that (−)-(1S,2S)-U50,488 is a potent KOR agonist, and at higher doses has activity both as a κ-ORA and sodium channel blocker.

In summary, we provide evidence in the present report that the sodium channel blocking actions of (−)-(1S,2S)-U50,488 and other arylacetamide κ-ORAs, including U50,488, could contribute to their peripheral as well as central antinociceptive actions. Such κ-ORAs, in a manner similar to local anesthetics such as lidocaine, produce a nonselective blockade of tetrodotoxin-sensitive as well as tetrodotoxin-resistant voltage-activated sodium currents in sensory neurons (S. K. Joshi, K. Bielefeldt, and G. F. Gebhart, unpublished data). In addition to analgesia, such interaction of κ-ORAs with sodium channels also has implications in the side effect profile of clinically used κ-ORAs, given the widespread distribution and critical role of sodium channels. For example, administration of κ-ORA can have effects on the cardiovascular system, like antiarrhythmia and hypotension on account of their sodium channel blocking actions on cardiac myocytes. Last, at a basic research level, these findings also provide additional structural information regarding the interaction of drugs with sodium channels.

References

Alzheimer C and Bruggencate G (1990) Nonopioid actions of the κ-opioid receptor agonists, U 50488H and U 69593 on electrophysiologic properties of hippocampal CA3 neurons in vitro. *J Pharmacol Exp Ther* **255**:900–905.
Bansinath M, Ramabadran K, Turndorf H, and Shukla VK (1991) (1991) Intracerebroventricular administration of κ-agonists induces convulsions in mice. *Brain Res Bull* **27**:75–79.
Joshi SK, Su X, Porreca F, and Gebhart GF (2000) (2000) κ-Opioid receptor agonists modulate visceral nociception at a novel, peripheral site of action. *J Neurosci* **20**:5874–5879.
Kolesnikov Y, Jain S, Wilson R, and Pasternak GW (1996) Peripheral κ1-opioid receptor-mediated analgesia in mice. *Eur J Pharmacol* **310**:141–143.
Millan MJ (1990) (1990) κ-Opioid receptors and analgesia. *Trends Pharmacol Sci* **11**:70–76.
Pugsley MK, Penz WP, Walker MJ, and Wong TM (1992a) Antiarrhythmic effects of U-50,488H in rats subject to coronary artery occlusion. *Eur J Pharmacol* **212**:15–19.
Pugsley MK, Penz WP, Walker MJ, and Wong TM (1992b) Cardiovascular actions of the κ-agonist, U-50,488H, in the absence and presence of opioid receptor blockade. *Br J Pharmacol* **105**:521–526.
Pugsley MK, Saint DA, Penz MP, and Walker MJ (1993) (1993) Electrophysiological and antiarrhythmic actions of the κ agonist PD 129290 and its R,R (+)-enantiomer, PD 129289. *Br J Pharmacol* **110**:1579–1585.
Pugsley MK, Saint DA, and Walker MJ (1994) An electrophysiological basis for the antiarrhythmic actions of the κ-opioid receptor agonist U-50,488H. *Eur J Pharmacol* **261**:303–309.
Pugsley MK, Yu EJ, and Goldin AL (2000) U-50,488H, a κ opioid receptor agonist, is a more potent blocker of cardiac sodium channels than lidocaine. *Proc West Pharmacol Soc* **43**:47–50.
Ramabadran K, Bansinath M, Turndorf H, and Puig MM (1988) Stereospecific inhibition of gastrointestinal transit by κ opioid agonists in mice. *Eur J Pharmacol* **155**:329–331.
Rothman RB, France CP, Bykov V, De Costa BR, Jacobson AE, Woods JH, and Rice KC (1989) Pharmacological activities of optically pure enantiomers of the κ opioid agonist, U50,488 and its cis diastereomer: evidence for three κ receptor subtypes. *Eur J Pharmacol* **167**:345–353.
Stein C, Machelska H, Binder W, and Schafer M (2001) Peripheral opioid analgesia. *Curr Opin Pharmacol* **1**:62–65.
Su X, Joshi SK, Kardos S, and Gebhart GF (2002) Sodium channel blocking actions of the κ-opioid receptor agonist U50,488 contribute to its visceral antinociceptive effects. *J Neurophysiol* **87**:1271–1279.
Su X, Julia V, and Gebhart GF (2000) Effects of intracolonic opioid receptor agonists on polymodal pelvic nerve afferent fibers in the rat. *J Neurophysiol* **83**:963–970.
Tallarida RJ and Murray RB (1991) *Manual of Pharmacological Calculations with Computer Programs*. Pharmacological Calculation System (Pharm/PC version 4.0), Springer Verlag, New York.
Taub DD, Eisenstein TK, Geller EB, Adler MW, and Rogers TJ (1991) Immunomodulatory activity of μ- and κ-selective opioid agonists. *Proc Natl Acad Sci USA* **88**:360–364.
Tjolsen A, Berge OG, Hunskaar S, Rosland JH, and Hole K (1992) The formalin test: an evaluation of the method. *Pain* **51**:5–17.
Wilson JL, Nayanar V, and Walker JS (1996) The site of anti-arthritic action of the κ-opioid, U-50,488H, in adjuvant arthritis: importance of local administration. *Br J Pharmacol* **118**:1754–1760.
Zhu Y and Im WB (1992) Block of sodium channel current by anticonvulsant U-54494A in mouse neuroblastoma cells. *J Pharmacol Exp Ther* **260**:110–116.

Address correspondence to: Dr. S. K. Joshi, Department of Pharmacology, BSB, The University of Iowa, Iowa City, IA 52242. E-mail: shailenjoshi@uiowa.edu