Putative kappa-2 Opioid Agonists Are Antihyperalgesic in a Rat Model of Inflammation

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Accepted for publication December 5, 1996

ABSTRACT

It has been demonstrated that kappa-2 opioid receptor agonists can inhibit the current that flows through the N-methyl-D-aspartate (NMDA) subclass of excitatory amino acid receptor. NMDA receptor antagonists have been shown to be effective antihyperalgesic agents when administered intrathecally into rats. Antihyperalgesia is defined as the ability to block enhanced sensitivity, usually produced by nerve injury or inflammation, to nociceptive stimuli. Thus, the hypothesis was proposed that kappa-2 opioid receptor agonists would be antihyperalgesic when injected intrathecally into rats with an inflamed hind paw. The kappa agonists bremazocine and GR89,696 were effective at reversing the hyperalgesia associated with the inflamed hind paw but did not influence the sensitivity of the noninflamed hind paw to noxious heat. The kappa-1-selective agonist U69,593 had no effect on the heat sensitivity of either the inflamed paw or the noninflamed paw. Intrathecal injection of the mu-selective agonist [D-Ala²,N-MePhe⁵,Gly⁵-ol]enkephalin or the delta-selective agonist [D-Pen²,⁵]enkephalin elevated paw withdrawal latencies to heat in both hind paws. These findings indicate that activation of presumed kappa-2 receptors in the rat spinal cord results in suppression of the hyperalgesic state without influencing normal sensitivity to noxious stimuli. It is proposed that the antihyperalgesic effect of kappa-2 receptor activation is mediated by the ability of the opioid receptor to reduce the flow of current through the NMDA receptor ionophore.

Inflammation or nerve injury often results in a state of heightened sensitivity to stimuli. If there is an increased perception of pain evoked by noxious stimuli, the damaged tissue is said to be hyperalgesic. Hyperalgesia is perhaps one of the most debilitating aspect of many injuries. The enhanced sensitivity to stimuli may make everyday tasks, such as putting on clothing, very difficult. Thus, effective treatments aimed specifically at hyperalgesia are desirable. Recent work with antagonists to the NMDA subclass of excitatory amino acid receptors indicates that these agents, when injected intrathecally, are antihyperalgesic (Dickenson, 1994; Eisenach and Gebhart, 1995; Ren et al., 1992a, 1992b; Tal and Bennett, 1994). An antihyperalgesic agent is one that blocks the enhanced sensitivity to noxious stimuli in injured tissue but does not influence normal sensitivity to noxious stimuli. NMDA receptor antagonists block nociceptive responses to noxious stimuli in damaged tissue. However, animals treated with intrathecal NMDA antagonists still respond normally to noxious stimulation of nondamaged tissue. Opioids, such as morphine, are analgesic as well as antihyperalgesic. These agents suppress nociceptive responses in both injured and uninjured tissue (Eisenach and Gebhart, 1995; Ren et al., 1992b). The advantage of the antihyperalgesic effect of NMDA antagonists over classic analgesics is that normal sensory function is spared. Thus, state-specific agents such as NMDA receptor antagonists are extremely attractive for pain control.

The mechanism by which NMDA receptor antagonists block hyperalgesia is beginning to be understood. Hyperalgesia is associated with an increased excitability of secondary neurons in the spinal cord. Central hyperexcitability is mediated, to a large extent, by NMDA receptors (Dickenson, 1994; Ren et al., 1992a). Blocking the NMDA receptors results in suppression of central hyperexcitability and, presumably, a decrease in hyperalgesia.

Recent work on in vitro hippocampal slices has demonstrated that a class of opioid receptor that has the characteristics of the kappa-2 receptor inhibits the flow of current through the NMDA receptor ionophore (Caudle et al., 1994). Because the kappa-2 receptor has not been cloned, the definition of this receptor remains operational. The receptor is defined as the naloxone-sensitive bremazocine binding that remains after mu, delta and kappa-1 receptors have been blocked with selective ligands (Nock et al., 1993). The kappa-1 receptors are defined as the binding sites occupied by U69,593 [N-methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide]. The term kappa-2 is used here because the kappa-selective opioid peptide dynorphin was dem-

ABBREVIATIONS: DAMGO, [D-Ala²,N-MePhe⁵,Gly⁵-ol]enkephalin; CFA, complete Freund’s adjuvant; DPDPE, [D-Pen²,⁵]enkephalin; NMDA, N-methyl-D-aspartate; ANOVA, analysis of variance.
onstrated to be an endogenous agonist for the receptor in the hippocampus (Caudle et al., 1994). The kappa-2 opioid receptor responds to the kappa agonists bremazocine, dynorphin (Caudle et al., 1994) and GR89,696 [methyl-4-[(3,4-dichlorophenyl)acetyl]-3-[[1-pyrrolidinyl]methyl]-1-piperazinecarboxylate] (Naylor et al., 1993) with a decrease in synaptically evoked NMDA receptor-mediated currents. The kappa-1 agonists U50,488 [3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide methanesulfonate] and U69,593 were without effect on this current. The mu and delta agonists DAMGO and DPDPPE enhanced the NMDA receptor-mediated current. These data indicate that in tissue in which the NMDA receptors and the kappa-2 opioid receptors are co-localized, the kappa-2 receptors can be used to reduce NMDA receptor activity. Therefore, because kappa-2 opioid receptors were shown to inhibit NMDA receptor-mediated currents and because NMDA receptor antagonists are known to be anti-hyperalgesic in a rat inflammation model, the hypothesis was proposed that intrathecally administered kappa-2 agonists would be anti-hyperalgesic rather than analgesic like classic opioids.

Methods

Male Sprague-Dawley rats (300–500 g) were implanted with intrathecal cannulas according to a modified method of Yaksh and Rudy (1977) as previously described (Caudle and Isaac, 1987). Briefly, the rats were anesthetized with either ketamine (50 mg/kg) and xylazine (10 mg/kg) or with halothane. An incision was made in the atlanto-occipital membrane, and a PE-10 cannula was inserted through the opening and guided to the top of the lumbar enlargement. The wound was then closed, and the rats were observed for 3 to 4 days for signs of motor impairment or infection before we began the experiments. Rats displaying any motor impairment were euthanized and not included in the study. Cannula placement was confirmed in a limited number of animals after the completion of the study. The rats were housed individually to prevent them from chewing on their cannulas.

At 24 hr before initiation of behavioral testing, the rats received an intraplantar injection of an emulsion of CFA and saline (1:1; 0.2 ml) into the right hind paw. This procedure produced a consistent inflammation and hyperalgesia to heat in the injected paw within 2 to 3 hr. The animal’s paws remained inflamed and hyperalgesic for several days after the CFA injection (Hylden et al., 1989). The injection did not influence the noninjected hind paw. The animals guarded the inflamed paw; however, they exhibited normal grooming behavior, gained weight, and moved about their cages with little difficulty. Hind paw withdrawal latencies from a radiant heat source were determined as previously described (Hargreaves et al., 1988) the day after the CFA injection. A cutoff time of 22 sec was used to prevent injury to the rat’s paws. Once stable withdrawal latencies were established, the rats received intrathecal injections of either opioids dissolved in 10 to 20 μl of saline or 10 μl of sterile saline through the implanted cannula. The cannulas were then flushed with 10 μl of sterile saline. Hind paw withdrawal latencies to heat were determined at 10 min after the intrathecal injection. Preliminary studies have indicated that this time point was within the range for the peak effect of all drugs used in this study. These procedures have been reviewed and approved by the National Institute of Dental Research Animal Care and Use Committee. Treatment of the animals conformed to the ethical guidelines of the International Association for the Study of Pain (Zimmerman, 1983).

Paw withdrawal latencies were normalized to the base-line latency of the hind paw that did not receive an injection of CFA (control latency) by the formula: (Test paw withdrawal latency/control latency) × 100. This value is referred to as the percentage of base-line paw withdrawal latency. Using this value, a score of 100 represents no hyperalgesia, a score of <100 represents hyperalgesia and a score of >100 is analgesia. ANOVAs and nonlinear regressions were performed on the normalized data using the statistical program PRISM (GraphPAD Software, San Diego, CA), and the Newman-Keuls test was used for post hoc analysis when necessary.

DAMGO, DPDPPE, naloxone and CFA were purchased from Sigma Chemical (St. Louis, MO). U69,593 and bremazocine were purchased from Research Biochemicals (Natick, MA). GR89,696 was a generous gift from Dr. B. M. Bain (Glxco, Middlesex, UK). All drugs were dissolved in 0.9% saline.

Results

At 24 hr after the intraplantar injection of CFA, the paw withdrawal latencies for the inflamed and noninflamed paws were 3.58 ± 0.06 and 10.03 ± 0.14 sec (mean ± S.E.M. for 378 animals, which was the total number of animals used in the study), respectively. Normalization of this data to percentage of base-line paw withdrawal latency provided a score of 37.47 ± 0.61 for the inflamed paw. The noninflamed paw before an intrathecal injection has a score of 100 by definition.

Intrathecal injection of the kappa agonist bremazocine elevated the score of the inflamed paw in a dose-dependent manner (ANOVA, dF = 49, F = 5.193, P = .0271) (fig. 1). The highest dose that could be dissolved in 20 μl of saline was 540 nmol. In contrast to the inflamed paw, the noninflamed paw was not influenced by bremazocine (ANOVA, dF = 49, F = .006, P = .94). The dose-response relationship for the kappa agonist GR89,696 was similar to that of bremazocine, in which the paw withdrawal score of the inflamed paw was elevated in a dose-dependent manner (ANOVA, dF = 48, F = 13.765, P = .0005) (fig. 2). GR89,696 was substantially more potent than bremazocine. The dose required to reverse hy-

\[ \text{Percentage Normalized Paw Withdrawal Latency} = \frac{\text{Paw Withdrawal Latency}}{\text{Control Latency}} \times 100 \]

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1 R. M. Caudle, unpublished observations.

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Fig. 1. Dose-response relationship for intrathecal bremazocine on paw withdrawal latencies in rats with CFA induce inflammation. Paw withdrawal latencies to heat for inflamed and noninflamed hind paws were determined before and 10 min after the intrathecal injection of bremazocine. The data were then normalized as described in Methods. Each point represents the mean of normalized paw withdrawal latencies for 4 to 16 rats. Error bars represent the S.E.M. Lines were fitted by nonlinear regression. Each rat only received one dose of bremazocine (ANOVA: inflamed paw, dF = 49, F = 5.193, P = .0271; noninflamed paw, dF = 49, F = .006, P = .94).
peralgesia by 50% (ED$_{50}$) was 3.7 ± 1.9 nmol, whereas the ED$_{50}$ for bremazocine was 54.6 ± 2.2 nmol. As with bremazocine, GR89,696 did not significantly raise the score on the inflamed paw above 100, nor did it influence the withdrawal score of the noninflamed paw (ANOVA, dF = 48, F = 13.765, P = .0005; noninflamed paw, dF = 48, F = .683, P = .4127). As illustrated in figure 3, the effects of GR89,696 could be blocked by a systemic injection of naloxone (1 mg/kg intraperitoneal) 15 min before injection of GR89,696. This indicates that the antihyperalgesic effect of GR89,696 was probably mediated through an opioid receptor. However, intraperitoneal naloxone could not reverse the antihyperalgesic effects of GR89,696 when injected 30 min after GR89,696. The time course of the antihyperalgesic action of GR89,696 was followed out to 24 hr. The effect of GR89,696 reached a peak within 10 min and the duration of action exceeded 3 hr (ANOVA, dF = 29, F = 7.612, P = .0003) (fig. 4). The drug also appeared to have an antihyperalgesic effect at 24 hr after administration. However, the hyperalgesia may have begun to resolve by this time (Hylden et al., 1989), masking the recovery from the effects of GR89,696. In a preliminary study, the paw withdrawal score for the inflamed paws of animals receiving intrathecal saline increased from 32.3 ± 6.7 on the day of the intrathecal injection to 58.3 ± 14.4 at 24 hr after the injection.

In contrast to the effects of the kappa agonists bremazocine and GR89,696, the kappa-1-selective agonist U69,593 had no effect on the withdrawal latencies of either the inflamed hind paw or the noninflamed hind paw. The paw withdrawal score for the inflamed paw after 180 nmol of U69,593 was 37.8 ± 5.3 (mean ± S.E.M., n = 6), whereas the score for saline-injected rats was 33.4 ± 4.7 (n = 6). This dose represents the maximum soluble dose of U69,593 that can be administered in 20 μl of saline. The paw withdrawal scores for the noninflamed paws after U69,593 and saline were 97.1 ± 9.2 and 90.6 ± 7.6, respectively.

To test the effect of mu or delta receptor activation on paw withdrawal latency in the rat inflamed paw model, various
Fig. 4. Time course for the antihyperalgesic action of GR89,696. Paw withdrawal latencies were measured before and 10 min, 60 min, 3 hr and 24 hr after the intrathecal injection of 3 nmol of GR89,696 into rats with unilateral hind paw inflammation. The data were normalized as described in Methods. The recovery from the antihyperalgesic effect of GR89,696 at the 24-hr time point may have been masked by the normal resolution of the hyperalgesia (Hylden et al., 1989). Error bars represent the S.E.M. * P < .05 by ANOVA followed by Newman–Keuls test when compared with the preinjection group.

Fig. 5. Dose-response relationship for DAMGO on paw withdrawal latencies in rats with CFA-induced inflammation. Paw withdrawal latencies were determined before and 10 min after the intrathecal injection of DAMGO. The data were then normalized as described in Methods. Each rat only received one dose of DAMGO (ANOVA: inflamed paw, dF = 34, F = 22.675, P = .0001; noninflamed paw, dF = 34, F = 17.312, P = .0002) (fig. 5). Like the mu agonist DAMGO, the delta-selective opioid agonist DPDPE produced a dose-dependent increase in the paw withdrawal scores for both the inflamed and noninflamed hind paws (ANOVA: inflamed paw, dF = 25, F = 24.453, P = .0001; noninflamed paw, dF = 25, F = 7.256, P = .0124) (fig. 6). However, the analgesic effects of DPDPE were not, in comparison with DAMGO, particularly robust. The maximum dose of DPDPE that could be dissolved in 20 μl of saline (300 nmol) elevated the inflamed paw withdrawal score to only ~100 and the noninflamed paw withdrawal score to 150. The maximum dose of DAMGO tested (30 nmol), on the other hand, increased paw withdrawal scores for both paws to ~250 (fig. 5).

Discussion

Several studies have demonstrated the potential of the use of NMDA receptor antagonists as state-specific agents for attenuating or blocking hyperalgesia (Dickenson, 1994; Eisenach and Gebhart, 1995; Ren et al., 1992a, 1992b; Tal and Bennett, 1994). In the present study, we demonstrated that the kappa agonists bremazocine and, particularly, GR89,696 are specific inhibitors of hyperalgesia in the rat model of peripheral inflammation when administered intrathecally. These drugs reverse the hyperalgesia associated with inflammation but do not influence the response of the animal's noninflamed paw to stimuli that would normally be considered noxious. Bremazocine was substantially less potent than GR89,696, and its dose-response relationship was shallower. Bremazocine was previously demonstrated to have agonist and antagonist actions at opioid receptors other than kappa receptors (Valeri et al., 1995); thus, its effects are not likely to be due to a single receptor. GR89,696, on the other hand, has greater selectivity for kappa receptors than bremazocine, and therefore its dose-response relationship may be more representative of selective kappa-2 receptor activation. The kappa-1 agonist U69,593 at the maximum soluble dose did not influence hyperalgesia in this model. The results with U69,593 are consistent with previous findings with intrathecal kappa-1-selective agonists (Danzebrink et al., 1995; Schmauss and Yaksh, 1984). .

Studies of kappa opioid receptor binding in rat spinal cord

\[ \text{Log Dose DAMGO (mols)} \]

[Graph showing log dose DAMGO against percentage baseline paw withdrawal latency]

\[ \text{Log Dose DPDPE (mols)} \]

[Graph showing log dose DPDPE against percentage baseline paw withdrawal latency]
have been controversial. Using [³H]-U69,593, Besse et al. (1992) demonstrated few kappa binding sites in the rat spinal cord. The Bₘₐₓ values for kappa, mu and delta receptors in the spinal cord found by this group were 11.8 ± 1.3, 65.0 ± 2.4 and 24.7 ± 2.0 fmol/mg of protein, respectively. This finding is consistent with behavioral studies in which intra-thecally injected kappa-1-selective agonists have little effect on nociceptive responses (Danzébrink et al., 1995; Schmauss and Yaksh, 1984). Iadarola et al. (1988), on the other hand, used [³H]bremazocine and selective mu and delta blocking ligands to demonstrate that kappa receptors were extremely abundant in rat spinal cord. These authors found that the Bₘₐₓ values for spinal kappa, mu and delta receptors were 105.5 ± 20.4, 32.4 ± 4.9 and 11.9 ± 1.3 fmol/mg of protein, respectively. It has since been demonstrated that bremazocine binds to more than one type of kappa opioid receptor (Nock et al., 1993), whereas U69,593 binds to a single type of receptor. Thus, the differences observed in spinal cord kappa receptors suggest that Iadarola et al. (1988) were considering kappa-1 and kappa-2 opioid receptors, whereas Besse et al. (1992) had limited their study to kappa-1 opioid receptors. Thus, a comparison of these studies suggests that there are a relatively large number of kappa-2 opioid receptors in the rat spinal cord. Despite attempts by many researchers, kappa-2 opioid receptors have not yet been cloned, nor have selective ligands been developed for this receptor. Thus, the definition of the kappa-2 receptor remains an operational label based on the unique binding (Nock et al., 1993; Tiberi and Magnan, 1990; Wood et al., 1989) and agonist/antagonist profile (Caudle et al., 1994) of this site. It remains to be determined whether this site is truly a unique opioid receptor, an unusual variant of a previously cloned receptor or an entirely new class of receptor.

In contrast to bremazocine and GR89,696, the mu-selective agonist DAMGO and the delta-selective agonist DPDPE were typical analgesics, inhibiting the response of the animals to stimulation of their noninflamed paw as well as to their inflamed paw. The profile of action of these opioid agonists and the sensitivity of GR89,696 to antagonism by naloxone suggest that bremazocine and GR89,696 act through a kappa-2 type of receptor (Caudle et al., 1994) to produce their antihyperalgesic effect. The kappa-1-selective antagonist norbinaltorphimine (Nock et al., 1993) was not used in this study because its selectivity has been unreliable in vivo (Guirmand et al., 1994). In addition, because U69,593 had no effect on inflammation-induced hyperalgesia, there was no positive control to verify that norbinaltorphimine was blocking kappa receptors. Thus, the use of norbinaltorphimine would have had little informational value. One particularly interesting finding was that naloxone could block the effects of GR89,696, but it could not reverse the effects of GR89,696. This finding is similar to in vitro results and suggests that a series of events are initiated after receptor activation that continue long after the receptor is no longer occupied by agonist. However, the post-GR89,696 naloxone treatment group tended to urinate a great deal, which made testing of paw withdrawal from heat difficult. The significant elevation of the withdrawal score of the noninflamed paws (fig. 3) suggests that the animals’ wet feet may have elevated the withdrawal latencies. This might have masked the antagonism by naloxone of GR89,696. Frequent urination was not a problem with the other treatment groups.

The mechanism by which kappa-2 opioid receptors inhibit hyperalgesia is not entirely certain. However, whole-cell patch-clamp studies in the CA3 region of the guinea pig hippocampus demonstrated that kappa-2 opioid receptor activation by bremazocine (Caudle et al., 1994) and GR89,696⁻¹ inhibited NMDA receptor-mediated synaptic currents. The fact that these agents are antihyperalgesic is consistent with their ability to regulate NMDA receptor function. Given the proposed role of NMDA receptors in hyperalgesia (Ren et al., 1992a, 1992b), it is likely that the kappa-2 opioid receptors are producing their antihyperalgesic effect by inhibiting NMDA receptors. This mechanism, however, will have to be verified through electrophysiological experiments in the spinal cord.

Notwithstanding the caveats outlined above for the kappa-2 opioid receptor, it is apparent that this site represents an extremely promising target for the pharmacological management of hyperalgesia. In addition, the concept of using antihyperalgesic agents rather than analgesic agents represents a significant refinement in pain control strategies.

Acknowledgments

We thank Drs. Eli Eliav and Michael Iadarola for their comments and advice in the preparation of the manuscript.

References


3 R.M. Caudle, unpublished observations.


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