Effects of Kappa-Opioid Receptor Agonists on Responses to Colorectal Distension in Rats with and without Acute Colonic Inflammation

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ABSTRACT

The objective of this study was to evaluate the effects of kappa-opioid receptor agonists on pressor and visceromotor responses to colorectal distension in awake, unrestrained rats, a model of visceral pain. Because visceral pain can be enhanced in the presence of inflammation, the study was conducted in rats that had been given either intracolonic saline or 5% acetic acid 6 hr before drug administration. We developed a method of staircase colorectal distension as a means of obtaining stimulus-response functions over a short period of time. Kappa-opioid receptor agonists, given i.v. in a cumulative dose paradigm, dose-dependently attenuated both the pressor and visceromotor responses to colorectal distension. In addition, all drugs tested also increased response threshold. The rank order of potency of the drugs tested was: CI977 > U69,593 > U50,488 ≥ morphine ≥ EMD61,753 > ICI204,448. Effective doses of these drugs were antagonized by naloxone, but not by either of two kappa-opioid receptor-selective antagonists (nor-binaltorphimine and 2-(3,4-dichlorophenyl)-N-methyl-N-(1-[3-isothiocyanate phenyl]-2-[1-pyrrolidinyl]ethyl)-acetamide). Acute inflammation of the colon did not lead to changes in the potency of the agonists tested. The present results provide further evidence that kappa-opioid receptor agonists significantly attenuate visceral nociception and, in conjunction with other information, suggest that a peripherally restricted kappa-opioid receptor agonist would be therapeutically effective in relieving visceral pain.

With the development of selective κ-ORAs, there has been a resurgence of interest in their role in the modulation of nociception. The kappa-opioid receptor, along with mu- and delta-opioid receptors, has been localized on cell bodies in dorsal root ganglia, particularly small dorsal root ganglion cells associated with unmyelinated and thinly myelinated axons (Ji et al., 1995; Minami et al., 1995; Schafer et al., 1994). Kappa-opioid receptors are also present in superficial and deeper layers of the spinal cord, regions where primary afferent neurons terminate and in brain regions known to be involved in nociceptive processing (e.g., nucleus tractus solitarius, raphe nuclei, periaqueductal gray area and thalamus; George et al., 1994; Mansour et al., 1994, 1987; Tempel and Zukin, 1987).

The antinociceptive efficacy of κ-ORAs in visceral nociception appears to be dependent on peripheral and supraspinal, but not spinal sites of action. In anesthetized rats, the magnitude of the pressor response to noxious CRD was attenuated by κ-ORAs administered i.v. or intracerebroventricularly, but not intrathecally (Diop et al., 1994a, b). Similarly, in awake rats, κ-ORAs administered i.v. and intracerebroventricularly, but not intrathecally, have been shown to attenuate responses to noxious CRD and to increase the visceromotor threshold for response (Danzebrink et al., 1995; Harada et al., 1995). In addition, κ-ORAs, but not μ- or δ-ORAs, dose-dependently attenuate responses of pelvic nerve afferent fibers to noxious CRD, suggesting a peripheral site of action for κ-ORAs (Su et al., 1997b; Sengupta et al., 1996).

Inflammation often accompanies pain as a significant component of many diseases. The process of inflammation results in the synthesis and/or release of numerous chemical mediators. Many of these mediators are capable of modulating neuron activity, some by sensitizing, others by activating nociceptors. In this way, visceral inflammation can alter the sensations produced by noxious and nonnoxious stimuli (for review, see Mayer and Gebhart, 1994). Moreover, the potency of opioids has been reported to increase in the presence of inflammation (for review, see Stein, 1993).

ABBREVIATIONS: CRD, colorectal distension; HAc, acetic acid; κ-ORA, kappa-opioid receptor agonist; MAP, mean arterial pressure; ΔMAP, change in mean arterial pressure; VMR, visceromotor response; EMG, electromyographic; SRF, stimulus-response function; DIPPA, 2-(3,4-dichlorophenyl)-N-methyl-N-(1-[3-isothiocyanate phenyl]-2-[1-pyrrolidinyl]ethyl)-acetamide; nor-BNI, nor-binaltorphimine.
Accordingly, the objective of our study was to examine the effects of systemically administered \(\kappa\)-ORAs on the pressor and visceromotor responses to nonnoxious and noxious intensities of CRD in the absence and presence of acute colonic inflammation. A preliminary report of some of these data has appeared in abstract form (Burton and Gebhart, 1995a).

**Methods**

Experiments were performed on unanesthetized rats 6 hr after intracolonic treatment with either 5% HAc or saline. The HAc model of acute colonic inflammation (MacPherson and Pfeiffer, 1978) was chosen because it mimics some clinical features of inflammatory bowel disease: edema, infiltrating leukocytes and increased content of mucosal eicosanoids (Fretland et al., 1990; Lauritsen et al., 1988).

Pressor and visceromotor responses (abdominal and hindlimb muscle contraction) to CRD were simultaneously recorded. These responses to CRD have been characterized and shown to require supraspinal integration (Ness and Gebhart, 1988a).

**Surgical preparation.** Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 340 to 580 g were anesthetized with pentobarbital sodium 50 mg/kg i.p. (Nembutal, Abbott Laboratories, North Chicago, IL). Femoral venous and arterial catheters were placed for drug administration and blood pressure recording, respectively, and multistranded, Teflon-insulated, 40-gauge, stainless-steel wires (Cooner Wire Co., Chatsworth, CA) were sutured into the external oblique musculature, just above the inguinal ligament, for EMG recordings. The catheters and EMG electrodes were subcutaneously guided to the dorsum of the neck and externalized for future access. The animals were housed separately with food and water ad libitum for a minimum of 3 days before experimentation.

**CRD.** On the day of testing, a 6- to 8-cm latex balloon (condom) tied to tygon tubing which extended into the balloon approximately 6 cm was lubricated with Surgilube (E. Fougera and Co., Melville, NY) and inserted into the colon via the anus. With the end of the balloon positioned 1 cm inside the rectum, the flexible catheter was taped to the base of the tail to prevent displacement. The catheter was then connected to a pressure control device (Bioengineering, North Chicago, IL). Femoral venous and arterial catheters were placed for drug administration and blood pressure recording, respectively, and multistranded, Teflon-insulated, 40-gauge, stainless-steel wires (Cooner Wire Co., Chatsworth, CA) were sutured into the external oblique musculature, just above the inguinal ligament, for EMG recordings. The catheters and EMG electrodes were subcutaneously guided to the dorsum of the neck and externalized for future access. The animals were housed separately with food and water ad libitum for a minimum of 3 days before experimentation.

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Rats were permitted movement as desired throughout testing. The balloon was inflated at 4-min intervals using a staircase paradigm. Starting at 0 mmHg, intracolonic pressure was incremented in approximately 10 mmHg steps over about 80 sec to a final pressure of 80 mmHg (fig. 1). Each pressure step was maintained for about 10 sec. Changes in MAP and in the visceromotor response (EMG) were quantified during the initial 5 sec of each distension step. This paradigm permits evaluation of drug effects on responses to both nonnoxious (≤30 mmHg) and noxious intensities of CRD and on pressor and visceromotor response thresholds.

Three to five staircase distensions, at 4-min intervals, were given 6 hr after intracolonic instillation of either saline or 5% HAc to establish predrug baseline response magnitude and threshold. Drugs were then administered i.v. using a cumulative dosing paradigm; two staircase distensions were given 4 and 8 min after drug administration, followed by the next dose of drug. Because agitation was produced in some rats by high doses of \(\kappa\)-ORAs, it was not always possible to test effects on CRD.

**Tissue inflammation.** At the end of each experiment the colon was examined visually for signs of inflammation. Signs of erythema, edema and deep focal lesions were noted and the inflammation was categorized as mild, moderate or severe.

**Drugs.** Appropriate volumes of drug solutions were administered through the i.v. catheter followed by an 80 μl flush of 0.9% saline. U50,488 (Research Biochemicals Inc. (RBI), Natick, MA) was dissolved in distilled water to a concentration of 16 mg/ml. U69,593 (RBI) was dissolved in 0.1 N HCl to a concentration of 25 mg/ml and diluted in saline to a final concentration of 5 mg/ml. CI977 (a generous gift from P. Boden, Parke-Davis Neuroscience Research Centre, Cambridge, England) was dissolved in saline to a concentration of 0.2 mg/ml. ICI204,448 (RBI) was dissolved in 0.1 M sodium carbonate to a concentration of 25 mg/ml. EMD61,753 (a generous gift from A. Barber, E. Merck, Darmstadt, Germany) was dissolved in distilled water by warming and vortexing to a concentration of 16 mg/ml and diluted in saline to a final concentration of 1.6 mg/ml. Morphine sulfate was dissolved in saline to a concentration of 12 mg/ml. Naloxone HCl was dissolved in saline to a concentration of 2 mg/ml. Nor-BNI (RBI) and DIPPA (Tocris-Cookson, St. Louis, MO) were prepared just before use in 0.5 ml saline.

**Data analyses.** Differences between resting EMG activity and MAP before and after drug administration were compared using a nonparametric Wilcoxon test to determine whether there were changes in these parameters attributable to drug action.

Dose-response relationships were constructed to show dose-dependent changes in response magnitudes at innocuous (20 mmHg) and noxious (60 mmHg) intensities of distension. ED\(_{50}\), defined as the dose that reduced the magnitude of the visceromotor and the pressor responses to 50% of pre-drug maximums, were determined from individual dose-response curves by linear regression. Comparisons of the ED\(_{50}\)s were made using Student’s t test.

Statistical tests were carried out using Minitab (State College, PA); P < .05 was considered significant.

**Results**

**Staircase CRD.** Staircase CRD produced graded pressor and visceromotor responses similar to those produced by phasic CRD (fig. 1; Burton and Gebhart, 1995b; Ness and Gebhart, 1988a). The approximate threshold for both re-

![Fig. 1. Example of visceromotor (VMB) and pressor (MAP) responses to staircase CRD (0 → 80 mmHg). The top panel represents the integrated EMG recorded from the external oblique musculature (derived from the raw EMG signal). MAP is displayed in the second panel. The bottom panel reflects the intracolonic pressure measured and maintained by the pressure controller. Shaded regions indicate the first 5 sec of each pressure step from which the responses to CRD were quantified.](image-url)
spontaneous responses is near 20 mmHg; responses are maximal and typically plateau at 60 to 80 mmHg (see also fig. 2). When distension is terminated, both EMG activity and MAP return to resting levels. Thus, as in studies using phasic CRD, responses to staircase CRD are graded and directly linked to the stimulus.

We developed the staircase method of CRD to permit evaluation of drug action on response threshold and on response magnitude. Figure 2 illustrates that SRFs, determined before and after i.v. administration of saline, are consistent between trials. There is some enhanced responsiveness to repeated CRD at greater intensities, consistent with previous studies using repeated phasic distension (Ness and Gebhart, 1988a, b; Ness et al., 1990; Burton and Gebhart, 1995b). Table 1 presents resting EMG activity and MAP for all rats in this study. In this protocol there was no significant difference in either resting EMG activity or MAP between saline- and HAc-treated animals.

**Inflammation.** Inflammation evaluated 6 hr after 5% HAc was mild in 44%, moderate in 29% and severe in 16% of the rats and typically included one-half of the length of the descending colon/rectum. No signs of inflammation were observed in 11% of the rats treated with HAc, nor in any of the rats treated with saline.

**Overview of drug effects.** Morphine, used as the prototype opioid, as well as all κ-ORAs tested were administered i.v. and all produced dose-dependent attenuation of both of the measured responses to CRD. The VMR and ΔMAP were similarly affected over the same dose range by morphine and κ-ORAs. Because drug effects were examined at intensities of CRD between 10 and 80 mmHg, modification of responses to an innocuous (20 mmHg) as well as a noxious (60 mmHg) intensity of CRD could be compared. κ-ORAs were also tested in animals with acutely inflamed or uninflamed colons. Drugs were found to be effective at inhibiting responses to innocuous and noxious intensities of CRD and similarly effective in rats with uninflamed or inflamed colons. Drugs were found to be effective at inhibiting responses to innocuous and noxious intensities of CRD and similarly effective in rats with uninflamed or inflamed colons. In general, κ-ORAs did not have a significant effect on resting EMG activity or MAP. However, in HAc-treated rats, U69,593 and ICI204,448 significantly decreased resting EMG activity (see table 1). In no case did any κ-ORAs produce flaccidity or motor impairment (as is often seen after intrathecal administration). However, most of the κ-ORAs produced agitation in the rats, characterized by vocalization, stretching or hopping behavior. These effects were produced in the mid- to upper-range of doses tested, which precluded the testing of greater drug doses, and were receptor-mediated (i.e., were antagonized by naloxone). The effects were also more pronounced in rats that received the centrally acting compounds as opposed to those that received compounds with restricted access to the central nervous system.

**Effects on VMR.** Two standard benzacetamide κ-ORAs (U50,488 and U69,593) and a novel, more potent benzacetamide (CI977) produced dose-dependent rightward shifts in the SRFs to CRD (fig. 3). Overall, VMR thresholds were significantly increased in a dose-dependent manner without significant effect on the slopes of the SRFs. Although CI977 produced an increase in response threshold similar to the other benzacetamides, it did not produce as great an attenuation of the VMR to 80 mmHg distension in saline-treated rats as did the other agonists. Morphine produced dose-dependent effects in both saline- and HAc-treated rats, similar to those produced by U50,488 and U69,593 (fig. 4).

Two newer, peripherally restricted κ-ORAs (ICI204,448 and EMD61,753) were also tested. These agonists also produced increases in response thresholds and attenuation of VMR magnitudes to CRD (fig. 4), but their effects were generally less than produced by agonists with greater access to the CNS. ICI204,448 produced similar effects in both saline- and HAc-treated groups. EMD61,753 produced more modest changes than any of the other κ-ORAs tested.

**Effects on VMR thresholds.** Linear regressions were performed on individual SRFs before and after each dose of drug and from it a pressure threshold for the VMR to CRD was extrapolated. The summary dose-response relationships from these analyses are plotted in figure 5. All κ-ORAs, including the peripherally restricted compounds, produced a significant increase in the threshold for response to CRD in a dose-dependent manner. This was true for both intracolonic saline- and HAc-treated groups. The dose-response relationships in the HAc-treated groups appear to be to the left of their saline-treated counterparts for most agonists (including morphine, but not U50,488 and ICI204,448). This would suggest that these drugs are more potent in altering the VMR threshold to CRD in HAc-treated animals than in saline-treated animals. There were, however, no statistically significant differences in dose-response relationships for any drug between saline- and HAc-treated groups. There was also a striking parallelism among the dose-response relations of all agonists.

**Effects on VMR magnitude.** Dose-response relationships were constructed to more clearly illustrate the dose-dependent effects of κ-ORAs on VMR magnitudes and to determine ED_{50}. As illustrated in figure 6, there is a dose-dependent reduction in the magnitude of the VMR to innocuous CRD (20 mmHg) with all κ-ORAs and morphine, and this occurs in saline- and HAc-treated animals. All κ-ORAs were able to completely inhibit the response to this innocuous intensity of CRD over the dose range examined. Figure 6 also presents the dose-response relationships at 60 mmHg, a noxious intensity of CRD. Morphine and all κ-ORAs dose-depen-
dently attenuated these responses as well. However, over the same dose range, the responses to noxious CRD were not completely inhibited, although the maximum effect was similar among the \(\kappa\)-ORAs and morphine as well as between saline- and HAc-treated groups.

**Effects on \(D\) MAP.** As presented in figures 7 and 8, the two standard benzacetamide \(\kappa\)-ORAs (U50,488 and U69,593), the novel benzacetamide (C1977), the peripherally restricted compounds and morphine all produced dose-dependent rightward shifts in the SRFs and attenuation of response magnitudes to CRD. The effects produced by EMD61,753 and to a lesser extent ICI204,448 seemed to be more bimodal than dose dependent (see fig. 8). Attenuation of the pressor response to CRD appears to be greater than that produced with respect to the VMR. On occasion, the \(D\) MAP in response to CRD after drug treatment converted to a depressor response at lower pressures of distension. Response magnitude, however, fits a linear SRF at the greater distending pressures. Therefore, the absolute change in MAP was taken as the response to CRD in all cases. The thresholds for \(D\) MAP in response to CRD were not affected by agonists to the same extent as were the VMR thresholds. Again, no differences were seen between saline- or HAc-treated groups. The sample sizes for changes in MAP are smaller for most of the agonists tested due to technical difficulties in maintaining patent arterial lines (and were incomplete for CI977 and ICI204488 in HAc-treated rats, fig. 8).

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Drug dose</th>
<th>MAP (mmHg)</th>
<th>EMG Activity (arbitrary units)</th>
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<tbody>
<tr>
<td></td>
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<td>Pre-drug</td>
<td>Post-drug</td>
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<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Saline</td>
<td>2 mg/kg</td>
<td>136.9 ± 2.1 (7)</td>
<td>127.9 ± 2.5 (7)</td>
</tr>
<tr>
<td>U50,488</td>
<td>200 (\mu)g/kg</td>
<td>129.3 ± 1.8 (6)</td>
<td>120.4 ± 3.1 (6)</td>
</tr>
<tr>
<td>U69,593</td>
<td>10 (\mu)g/kg</td>
<td>132.9 ± 2.3 (3)</td>
<td>113.0 ± 9.9 (3)</td>
</tr>
<tr>
<td>CI1977</td>
<td>10 (\mu)g/kg</td>
<td>129.5 (2)</td>
<td>123.3 (2)</td>
</tr>
<tr>
<td>EMD61,573</td>
<td>2 mg/kg</td>
<td>138.2 ± 1.9 (4)</td>
<td>133.5 ± 2.0 (4)</td>
</tr>
<tr>
<td>Morphine</td>
<td>1 mg/kg</td>
<td>135.1 ± 8.4 (3)</td>
<td>117.0 ± 1.1 (3)</td>
</tr>
<tr>
<td>5% HAc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U50,488</td>
<td>2 mg/kg</td>
<td>125.8 ± 9.3 (3)</td>
<td>123.4 ± 8.9 (3)</td>
</tr>
<tr>
<td>U69,593</td>
<td>200 (\mu)g/kg</td>
<td>129.8 ± 7.2 (4)</td>
<td>116.4 ± 8.7 (4)</td>
</tr>
<tr>
<td>CI1977</td>
<td>10 (\mu)g/kg</td>
<td>134.7 (2)</td>
<td>127.4 (2)</td>
</tr>
<tr>
<td>EMD61,573</td>
<td>10 (\mu)g/kg</td>
<td>126.1 (1)</td>
<td>122.6 (1)</td>
</tr>
<tr>
<td>Morphine</td>
<td>1 mg/kg</td>
<td>141.5 ± 1.3 (3)</td>
<td>130.8 ± 8.5 (3)</td>
</tr>
<tr>
<td>EMD61,573</td>
<td>2 mg/kg</td>
<td>137.6 ± 3.1 (4)</td>
<td>126.8 ± 4.0 (4)</td>
</tr>
<tr>
<td>Morphine</td>
<td>1 mg/kg</td>
<td>141.5 ± 1.3 (3)</td>
<td>130.8 ± 8.5 (3)</td>
</tr>
</tbody>
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All data are expressed as mean ± S.E.M. (\(n\)).

* Significant differences between pre- and post-drug treatment.
Dose response relationships were constructed to determine effects of drugs on the ΔMAP produced by CRD. Because the pressor response threshold to CRD is near 20 mmHg (Ness and Gebhart, 1988a; Danzebrink and Gebhart, 1990), it was not possible to reliably determine effects of drugs on the ΔMAP at 20 mmHg CRD. For example, the mean magnitude of response to a 20 mmHg distension in the present study was 6.2 ± 0.5 mmHg before drug administration, which is an inadequate magnitude of response in an awake animal to study changes produced by drugs. However, the ΔMAP to noxious (60 mmHg) CRD was 21.7 ± 1.0 mmHg, a sufficiently large response that can be used to reliably study changes produced by drugs. Figure 9 illustrates the dose-dependent attenuation of responses to noxious (60 mmHg) CRD. The dose-response relationships are parallel among agonists and the maximum effect of all agonists was similar between saline- and HAc-treated animals.

Comparison of ED₅₀s. From individual dose response curves, ED₅₀ values were determined. The ED₅₀ was defined as the dose of drug producing a 50% reduction in response to CRD. This determination was made for responses to both innocuous (20 mmHg) and noxious (60 mmHg) CRD, as well as for both saline- and HAc-treated groups (fig. 10; table 2). For the ΔMAP produced by 60 mmHg CRD, the rank order potency for the agonists tested was: CI977 > U69,593 > U50,488 > EMD61,753 > morphine > ICI204,448 in both saline- and HAc-treated groups. In some instances it was not possible to determine individual ED₅₀s and an estimate was made instead from the population dose-response relationship. This was the case for CI977 and ICI204,448 in saline-treated rats.

Comparisons of the ED₅₀ for each agonist on the VMR indicate that κ-ORAs more potently attenuate responses to innocuous CRD. The difference in the ED₅₀s of ICI204448 and CI977 between innocuous and noxious CRD did not reach statistical significance, although the individual ED₅₀s were always 2- to 10-fold greater for noxious CRD. There were no significant differences in the ED₅₀s of drugs between saline- and HAc-treated rats except for U69,593, which was more potent in HAc-treated rats at both innocuous and noxious intensities of CRD, and EMD61,753 (based on estimated ED₅₀s), which was less potent in HAc-treated rats at the innocuous intensity (20 mmHg) of CRD.

Comparisons of the ED₅₀ for each agonist on ΔMAP indicate a similar pattern to that of ED₅₀s for the VMR with two exceptions: the ED₅₀s for U50,488 and U69,593 suggest that these agonists were more potent in attenuating the ΔMAP than the VMR to noxious CRD.
Antagonism of effects. Attempts were made to determine the receptor selectivity of the $\kappa$-ORAs. Low doses of naloxone (10 and 30 $\mu$g/kg), administered cumulatively at the end of an experiment (20 min after administration of the last dose of a $\kappa$-ORA), partially reversed $\kappa$-ORA-produced attenuation of the VMR to CRD [the data for different $\kappa$-ORAs (U50,488, U69,593, EMD61,753, ICI204,448) and the two intensities of CRD were pooled because there were no differences]. The VMR was attenuated by $\kappa$-ORAs to a mean 7.5 ± 2.5% of pre-drug control ($n = 37$), which was partially reversed by 10 and 30 $\mu$g/kg of naloxone to a mean 47.6 and 51.8% of control, respectively. In contrast, the effects of morphine were completely antagonized. Morphine produced a mean attenuation of the VMR to 7.0 ± 1.6% of pre-drug control ($n = 12$), which was reversed to 116% of control by 10 $\mu$g/kg of naloxone. The greatest dose of naloxone tested (100 $\mu$g/kg) completely antagonized the effects of the $\kappa$-ORAs (to 99.5% of pre-drug control; $n = 24$). Rats pre-treated with a low dose of naloxone did not exhibit antagonism of the effects of bolus ED$_{50}$ doses of $\kappa$-ORAs, indicating that $\kappa$-ORAs were not acting at $\mu$-opioid receptors.

There are two reportedly selective, noncompetitive $\kappa$-opioid receptor antagonists, nor-BNI and DIPPA. Rats were pretreated with doses of nor-BNI ranging from 4 to 20 mg/kg for 48 hr. Another group of rats were pretreated with 0.24 to 0.5 mg/kg DIPPA at times ranging from 4 hr to 6 days. None of these treatment schedules led to antagonism of the effects of ED$_{50}$ doses of $\kappa$-ORAs, indicating that $\kappa$-ORAs were not acting at $\mu$-opioid receptors.

Discussion

Three main findings derive from this study. First, staircase CRD is an efficient means of obtaining stimulus-response functions. Both the visceromotor and pressor responses to staircase CRD are easily quantified, reliable and reproducible. Accordingly, drug action can be determined with respect to both response threshold and response magnitude at innocuous and noxious intensities of CRD. Consequently, the model provides information regarding different parameters of responses to CRD and changes in these parameters produced by interventions in an expedient manner. Second, $\kappa$-ORAs dose-dependently attenuate the magnitude of re-
In our study, there was no apparent hindlimb flaccidity; this generally occurs after intrathecal drug administration.

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Kappa ORAs are antinociceptive not only in cutaneous nociception (Herraro and Headley, 1993; Leighton et al., 1990) and the δ-ORAs are also similar to those producing cutaneous antinociception (Herraro and Headley, 1993; Leighton et al., 1996; Su et al., 1997). In addition, because the δ-ORAs were not acting at kappa-opioid receptors to produce the antinociceptive effects reported here, then the ED50s and rank order potency would be expected to differ from those reported in H2 antagonist assays where the agonists are known to act through kappa-opioid receptors. The rank order potency in the present study is similar to that determined by others (Herraro and Headley, 1993; Hunter et al., 1990) and the ED50s are also similar to those producing cutaneous antinociception (Herraro and Headley, 1993; Leighton et al., 1998). In our study it is assumed that at least part of the effects of δ-ORAs are exerted through an action on receptors in the central nervous system. If an effective concentration of an antagonist is not achieved and maintained in the brain, this could explain the lack of substantial antagonism. Takemori et al. (1988) noted that systemically administered nor-BNI has difficulty in crossing the blood-brain barrier and required a significant latency to take effect centrally. This seems unlikely in our experiments, however, given that both nor-BNI and DIPPA were given, in different experiments, hours to days in advance of an experiment. Conversely, because the effects of κ-ORAs are also exerted through an action on receptors in the periphery, an effective concentration of antagonist may not have been achieved at the receptors in the colon/rectum. There is no published literature of which we

TABLE 2
ED_{50} for the visceromotor response (VMR) and ΔMAP to 60 mmHg CRD. All values are expressed in mg/kg. An ED50 for the ΔMAP was either estimated or was not obtained for all drugs tested.

<table>
<thead>
<tr>
<th>Drug</th>
<th>VMR</th>
<th>ΔMAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>HAc</td>
</tr>
<tr>
<td>CI977</td>
<td>0.01 ± 0.004</td>
<td>0.01 ± 0.004</td>
</tr>
<tr>
<td>U69,593</td>
<td>0.5 ± 0.03</td>
<td>0.2 ± 0.08</td>
</tr>
<tr>
<td>U50,488</td>
<td>1.8 ± 0.4</td>
<td>0.9 ± 0.2</td>
</tr>
<tr>
<td>Morphine</td>
<td>1.7 ± 0.3</td>
<td>2.2 ± 1.0</td>
</tr>
<tr>
<td>EMD61,753</td>
<td>2.1 ± 0.04</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>ICI204,448</td>
<td>8.1 ± 1.4</td>
<td>8.3 ± 2.0</td>
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* Significant difference between the VMR and ΔMAP ED50s within a treatment group.

Response and also increase response threshold to CRD. Third, the responses to CRD in rats with acutely inflamed and uninflamed colon are equally attenuated by κ-ORAs.

The receptor selective κ-ORAs tested here significantly attenuated responses to CRD at both innocuous and noxious intensities of distension, although also dose-dependently increasing response threshold. In addition, morphine produced the same effects. We are confident that these effects are antinociceptive and not related to either motor impairment or a change in compliance of colonic smooth muscle. Although κ-ORAs have been reported to produce paralysis (Borgbjerg et al., 1996; Leighton et al., 1988; Stevens and Yaksh, 1986), this generally occurs after intrathecal drug administration. In our study, there was no apparent hindlimb flaccidity; furthermore, the VMR and the ΔMAP were similarly affected by κ-ORAs. If flaccidity were to occur, the VMR and ΔMAP would be expected to be affected differently. The compliance of the colon is not changed by κ-ORAs in vivo (Burton MB and Gebhart GF, unpublished observations) and κ-ORAs also have no effect on smooth muscle contractility in vitro (Su et al., 1997b).

The κ-ORAs tested were effective in dose ranges similar to those previously reported by others in models of cutaneous nociception. Herraro and Headley (1991) found doses of morphine and U-50,488 similar to those used here to effectively inhibit the flexor reflex in response to noxious pinch. Hunter et al. (1990) reported that effective antinociceptive doses of U50,488, U69,593, and CI977 in a paw pressure test were similar to the doses effective in our study. In a model of visceral nociception, Harada et al. (1995) found that U50,488 affected the VMR threshold to CRD in rats in a dosage similar to that found effective here. In some models of cutaneous nociception, investigators concluded that κ-ORAs were selective for modality of stimulation (thermal nociceptive assays were considered less sensitive than mechanical nociceptive assays to κ-ORAs). However, Parsons and Headley (1998) and Dong et al. (1991) have shown that the efficacy of κ-ORAs is dependent on stimulus intensity and that κ-ORAs are not modality-selective. Our data further document that κ-ORAs are antinociceptive not only in cutaneous nociception, but also in a model of mechanical visceral nociception.

The κ-ORAs tested were uniformly more potent against responses to innocuous (20 mmHg) CRD than responses to noxious (60 mmHg) CRD. Studies of cutaneous nociception also show that opioids attenuate lesser intensity stimuli to a greater extent than greater intensity stimuli. Dong et al. (1991) reported that U50,488 attenuated spontaneous activity of convergent dorsal horn neurons to a greater degree than evoked responses of the same neurons. In addition, at any given dose there was a greater effect on responses to non-noxious than noxious intensities of stimulation.

Although nor-BNI (Takemori et al., 1988) and DIPPA (Chang et al., 1994) were ineffective in blocking the action of κ-ORAs in this study, a 100-μg/kg dose of naloxone produced complete antagonism. It is unclear why these kappa-opioid receptor-selective antagonists were ineffective. One possibility is that the agonists tested were not acting at kappa-opioid receptors. For example, Horan et al. (1991) noted that sedation produced by U69,593 and bremazocine was not reversed by the kappa-opioid receptor antagonist UPHIT whereas the antinociception produced by U69,593 was reversed by the antagonist. They suggested that the sedation was mediated by nonopioid receptor activation, although they did not test whether the effect could be reversed by naloxone. In our study it is unlikely that nonopioid receptors mediated the effects reported here, because a high, nonopioid receptor-selective dose of naloxone antagonized the effects of κ-ORAs on CRD. Because a low, μ-opioid receptor-selective dose of naloxone did not antagonize the effects of the κ-ORAs tested, it is unlikely that the κ-ORAs tested produced their effects at μ-opioid receptors. The same “negative” results have been obtained in experiments examining κ-ORA effects on responses of pelvic nerve afferent fibers to CRD (Sengupta et al., 1996; Su et al., 1997b). In addition, if κ-ORAs were not acting at kappa-opioid receptors to produce the antinociceptive effects reported here, then the ED50s and rank order potency would be expected to differ from those noted in antinociceptive assays where the agonists are known to act through kappa-opioid receptors. The rank order potency in the present study is similar to that determined by others (Herraro and Headley, 1993; Hunter et al., 1990) and the ED50s are also similar to those producing cutaneous antinociception (Herraro and Headley, 1993; Leighton et al., 1998). In our study it is assumed that at least part of the effects of κ-ORAs are exerted through an action on receptors in the central nervous system. If an effective concentration of an antagonist is not achieved and maintained in the brain, this could explain the lack of substantial antagonism. Takemori et al. (1988) noted that systemically administered nor-BNI has difficulty in crossing the blood-brain barrier and required a significant latency to take effect centrally. This seems unlikely in our experiments, however, given that both nor-BNI and DIPPA were given, in different experiments, hours to days in advance of an experiment. Conversely, because the effects of κ-ORAs are also exerted through an action on receptors in the periphery, an effective concentration of antagonist may not have been achieved at the receptors in the colon/rectum. There is no published literature of which we
are aware that addresses this possibility. A recent report does, however, document the cellular localization of the cloned kappa-opioid receptors in the gastrointestinal tract of the rat (Bagnol et al., 1997). A third alternative is that the kappa-like receptors mediating visceral antinociception are distinct and, although they recognize κ-ORAs, they do not have affinity for the antagonists. Support for this suggestion is provided by the observation that the ED_{50} for κ-ORAs determined in experiments where their effects have been examined on decentralized colonic afferent fibers are virtually the same, ranging between 2 to 10 mg/kg (Sengupta et al., 1996; Su et al., 1997b). The recent report of Bagnol et al. (1997) does not address the presence of opioid receptors on the terminals of the extrinsic innervation of the colon.

Most information regarding kappa-opioid receptor subtypes has been derived from in vitro binding-studies and supported by data obtained in antinociceptive assays (e.g., Clark et al., 1989; Kosterlitz et al., 1981; Zukin et al., 1988; see Rothman, 1994 for overview). It is considered that there are several kappa-opioid receptor subtypes, but the majority of antinociceptive studies to date support the existence of two principal receptor subtypes: κ\(_1\) and κ\(_2\). It is generally accepted that the arylacetamides such as U50,488 bind preferentially to κ\(_1\) receptors whereas the benzomorphan bind preferentially to κ\(_2\) receptors, but are less selective. It has, however, been reported that the arylacetamide CI977 is somewhat more resistant to blockade by nor-BNI than the other drugs in its class (Herraro and Headley, 1991). This was also noted to display a pharmacological profile somewhat different than its counterparts (Herraro and Headley, 1991; Butelman et al., 1993). We have shown that κ-ORAs attenuate pressor and visceromotor responses as well as primary afferent nerve responses to visceral nociception, yet in neither study were these effects capable of being antagonized by the kappa-opioid receptor antagonists available (present study and Sengupta et al., 1996; Su et al., 1997a,b). This may suggest that kappa-opioid receptors mediating visceral antinociception are a subtype that differs from those modulating cutaneous nociception.

The responses of rats with acutely inflamed colons were also attenuated by kappa-ORAs. The ED\(_{50}\) for inhibition of the VMR and ΔMAP were similar between saline- and HAc-treated groups for most agonists. This appears to conflict with the results reported by Langlois et al. (1994) who found that some κ-ORAs (PD117302 and fedotozine) were more potent in HAc-treated rats whereas U50,488 was equipotent. The responses measured in the study by Langlois et al. (1994) were changes in MAP in response to noxious CRD (100 mmHg) in anesthetized rats, 30 min after 0.6% intracolonic HAc-treatment. There are several factors that could explain the differences between studies. It is possible that the anesthetic unmasksa a difference not seen in the awake animal. In our study, 2 mg/kg U50,488 did not affect resting MAP or EMG activity whereas in the study of Langlois et al. (1994) a similar dose of U50,488 produced a fall in resting MAP of more than 40 mmHg. An alternative explanation is that in the proinflammatory process kappa-opioid receptors are more accessible or more sensitive to κ-ORAs. Recently it has been shown that inflammation creates a disruption of the perineural barrier that can facilitate the penetration of opioids to receptors on sensory nerves (Antonijevic et al., 1995). If this occurs, it could explain an increase in the potency of agonists. However, it would not explain why the potencies of only certain agonists would be increased.

The time course and/or concentration of the colonic irritant is likely important in evaluating modulation of nociception by κ-ORAs. Numerous studies reveal that opioids exhibit increased potency under conditions of inflammation. In fact, some investigations have reported κ-ORAs to be antinociceptive only during inflammation (Keita et al., 1995; Andreov et al., 1994; Idampaan-Heikila et al., 1994). Stein et al. (1988) reported that hindpaw inflammation enhances sensitivity of mechanical nociception to μ- and κ-ORAs, and that this is a peripherally mediated event. Later it was found that dynorphin (a kappa-opioid receptor-prefering endogenous ligand) was present in peripheral nerve fibers and immunocytes during inflammation (Hassan et al., 1992). In longer-term inflammation of the colon (4 days after trinitrobenzene sulfonic acid instillation into the colon), the ED\(_{50}\) of the peripherally restricted κ-ORA EMD61,753 was found to be significantly reduced (i.e., potency was increased; Snider et al., 1997). This outcome supports the notion that acute inflammation as studied here is inadequate for significant changes in kappa-opioid receptors to be produced after tissue insult.

In summary, κ-ORAs were shown in our study to dose-dependently attenuate pressor and visceromotor responses to innocuous and noxious intensities of CRD. In addition, the threshold for both responses was increased. Therefore, it is likely that κ-ORAs have a potential therapeutic value as analgesics, provided it is possible to prevent the undesirable side effects associated with activation of central nervous system kappa-opioid receptors.

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References


Diop L, Riviere PJM, Pascaud X, Dassaud M and Junien J-L (1994b) Role of vagal


Sengupta JN, Su X and Gebhart GF (1996) Kappa, but not mu or delta opioids attenuate responses to distension of pelvic nerve afferents innervating the colon of the rat. *Gastroenterology* **111:**968–980.


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