Effects of Intracolonic Opioid Receptor Agonists on Polymodal Pelvic Nerve Afferent Fibers in the Rat

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Su, X., V. Julia, and G. F. Gebhart. Effects of intracolonic opioid receptor agonists on polymodal pelvic nerve afferent fibers in the rat. J. Neurophysiol. 83: 963–970, 2000. We studied the effects of intracolonic administration of opioid receptor agonists (ORAs) on responses of pelvic nerve afferent fibers to colorectal distension (CRD) and heat. Single-fiber recordings were made from the decentralized S1 dorsal rootlet in the rat. An ~7-cm length of descending colon was isolated in situ to permit intracolonic perfusion with Krebs solution, which, when the outflow was clamped, was used to distend the colon. Responses to noxious CRD (40 mmHg, 30 s) were tested after intracolonic instillation of μ-, δ- or κ-ORAs. Intracolonic administration of the κ-ORAs EMD 61,753 (n = 5/12) and U62,066 (n = 8/11), but not either the μ-ORA fentanyl or the δ-ORA SNC-80, concentration-dependently inhibited responses of afferent fibers. For fibers unaffected by intracolonic administration of EMD 61,753 or U62,066, intra-arterial administration of κ-ORAs was effective. Forty-one of 54 mechanosensitive fibers also responded to intracolonic instillation of heated Krebs solution (50°C). Intra-arterial injection of fentanyl or mefenamic acid did not attenuate responses to heat. Either intracolonic or intra-arterial administration of κ-ORAs was effective. Forty-one of 54 mechanosensitive fibers also responded to intracolonic instillation of heated Krebs solution (50°C). Intra-arterial injection of fentanyl or SNC-80 did not attenuate responses to heat. Either intracolonic or intra-arterial administration of EMD 61,753 or U62,066, however, inhibited afferent fiber responses to heat. These results document that mechanical and thermal sensitivity of polymodal pelvic nerve afferent fibers innervating the rat colon can be inhibited peripherally by intracolonic instillation of κ-ORAs.

INTRODUCTION

Studies in humans and nonhuman animals have established that both nonpainful and painful sensations from the distal gut and urinary bladder are conveyed by the pelvic nerve to the spinal cord (e.g., Head 1893; White 1943; see Ness and Gebhart 1990 for review). We previously reported that κ-, but not either μ- or δ-opioid receptor agonists (ORAs) dose-dependently inhibit responses of mechanosensitive pelvic nerve afferent fibers to noxious colorectal or urinary bladder distension in the rat (Sengupta et al. 1996; Su et al. 1997a,b). Considerable efforts have been made to develop κ-ORAs with limited access to the CNS, where they produce unacceptable sedation and dysphoria (Barber et al. 1994; Rogers et al. 1992; Shaw et al. 1989). One such compound, EMD 61,753, is a peripheral restricted κ-ORA (Barber et al. 1994) that inhibits responses of pelvic nerve afferent fibers to colorectal distension (CRD) (Sengupta et al. 1999). Because these recordings were made in experiments in which pelvic nerve input was decentralized at the dorsal root, the site of action of the κ-ORAs was limited to the periphery, either at a peripheral receptor in the tissue, axon, and/or cell body in the dorsal root ganglion (DRG). Regarding the latter, we studied the effects of κ-, μ-, and δ-ORAs on high-voltage–activated calcium channels in colon sensory neurons, concluding that only part of the dose-dependent attenuation by κ-ORAs of responses to noxious CRD can be accounted for by an action at the level of the DRG (Su et al. 1998b). In the present study, we examined ORA effects on responses to noxious CRD, limiting effects of drugs to the periphery by intracolonic administration.

We have to date only examined κ-ORA effects on mechanical stimulation of the colon and bladder. It has been suggested that κ-ORAs are more effective against noxious mechanical stimuli than noxious heat stimulation (Abbott et al. 1986; Millan 1989, 1990; Tyers 1980). A recent report (Gschössmann et al. 1997) suggested that fedotozine, a drug with agonist efficacy at the κ-opioid receptor, inhibited a mechanically stimulated, but not capsaicin-stimulated Ca2+ increase in DRG cells, again suggesting modality selective actions of κ-ORAs. Other work, however, suggests that the reported relative selectivity of κ-ORAs against mechanical input is more related to stimulus intensity than to stimulus modality (e.g., Millan 1989; Parsons and Headley 1989a,b).

Because mechanosensitive pelvic nerve afferent fibers innervating the colon of the rat are activated by noxious chemical and/or thermal stimuli (i.e., are polymodal) (Su and Gebhart 1998b), the objectives of this study were twofold: 1) to examine the effects of intracolonic administration of ORAs on responses of mechanosensitive pelvic nerve afferent fibers to noxious CRD and 2) to examine the effects of ORAs on the responses of mechanosensitive pelvic nerve afferent fibers to noxious heat stimulation. Some of these data have been reported in abstract form (Su et al. 1998a).

METHODS

General procedures

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 410–530 g were used. Food, but not water, was withheld for 24 h before an experiment. Rats were anesthetized initially with 40–45 mg/kg ip pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL); anesthesia was maintained by infusion of pentobarbital (5–10 mg·kg−1·h−1 iv). A femoral artery and vein were catherized for measurement of arterial pressure and administration of drugs, respectively; the trachea was also cannulated to provide artificial ventilation. Rats were paralyzed with pancuronium bromide (initial 0.3 mg/kg iv; supplemental 0.2–0.3 mg·kg−1·h−1 iv). Core body temperature was maintained at 36°C by a hot-water-circulating heating pad placed under the rat and an overhead feedback-controlled heat lamp (thermoprobe inserted into the thoracic esophagus). At the end of experiments, rats were killed by an overdose of intravenous...
pentobarbital. The experimental protocol was approved by the Institutional Animal Care and Use Committee of The University of Iowa.

Surgical procedures

The surgical procedures have been described in detail (Su and Gebhart 1998a,b) and are only briefly summarized here. The lower abdomen was exposed by a 4- to 5-cm-long incision laterally at the left flank. An ~7 cm length of descending colon was exposed and isolated in situ. The blood supply and nerves innervating the colon remained intact. Both the proximal end and anus end of the descending colon were catheterized to permit intracolonic perfusion of the colon with Krebs-Henseleit (Krebs) solution. The pelvic nerve was isolated from the surrounding fatty tissues, and a pair of Teflon-insulated stainless steel wires were wrapped around the pelvic nerve and sealed with nonreactive Reprosil (hydrophilic vinyl polysiloxane, type 1; The L. D. Caulk Division, Dentsply International, Millford, DE). The abdomen was closed with silk sutures. The lumbarosacral spinal cord was exposed by laminectomy (T13–S2), the dura was carefully removed, and the spinal cord was covered with warm (37°C) mineral oil.

The isolated colon was connected to a pressurized fluid reservoir through the proximal catheter, and intracolonic pressure was measured through a fine catheter (polyethylene tubing, PE-60) placed in the colon from the proximal end. The pressure reservoir was connected to a distension control device via a low-volume pressure transducer (see Gebhart and Sengupta 1995). At rest, 37°C Krebs solution (0 mmHg) remained in the colon. For phasic, constant pressure distension (5–60 mmHg, 30 s), 37°C Krebs solution was introduced via the proximal catheter, and the distal catheter was clamped.

Thermal stimulation of the colon was produced by changing the temperature of the Krebs solution with which the isolated colon was perfused. In these experiments, the colon was continuously perfused with heated Krebs solution, and intracolonic pressure was ≤5 mmHg (i.e., perfusion pressure was 5 mmHg with the distal outflow open). To monitor the temperature of the perfusate, a thermoprobe (Physitemp, type IT-1E; Physitemp Instruments, Clifton, NJ) was introduced into the colon via the anal catheter. Thermal stimulation of the colon was produced by ramp increases in temperature (37–50°C, ~480 s) without changing intracolonic pressure while outflow was open.

Recording of afferent nerve action potentials

The S1 dorsal root was decentralized close to its entry to the spinal cord. Recordings were made from the distal cut end of the central processes of primary afferent fibers. The dorsal rootlet was split into thin bundles, and a fine filament was isolated to obtain a single unit. Electrical activity of the single unit was recorded by placing the teased fiber over one arm of a bipolar silver-silver chloride electrode; a fine strand of connective tissue was placed across the other pole of the electrode. Action potentials were monitored continuously by analogue delay and displayed on a storage oscilloscope after initial amplification through a low-noise AC differential amplifier. The action potentials were processed through a window discriminator and counted using the spike2/CED 1401 data acquisition program. Peristimulus time histograms (1-s binwidth), intracolonic pressure, intracolonic temperature, colonic distending pressures, and blood pressure were displayed on-line continuously.

Experimental protocol

CHARACTERIZATION OF AFFERENT FIBERS. Mechanosensitive pelvic nerve input to the S1 dorsal root was identified by electrical stimulation of the pelvic nerve (single pulse 0.5-ms square wave at 4–10 V) and CRD (40 mmHg, 3–5 s). Fibers with conduction velocities (CV) <2.5 m/s were considered unmyelinated C-fibers and those with CV >2.5 m/s were considered thinly myelinated Aδ-fibers.

Stimulus-response functions (SRFs) to phasic distending pressures of 5, 10, 20, 30, 40, and 60 mmHg, 30 s at 4-min intervals were determined for all fibers. Thermosensitivity was evaluated for 54/70 fibers by recording activity while increasing intracolonic temperature.

EFFECTS OF INTRACOLONIC ADMINISTRATION OF ORAS ON MECHANONOCICEPTION. The effects of ORAs were tested on responses of afferent fibers to 40 mmHg CRD. The volume of Krebs solution that produced 40 mmHg distension was 5.2–5.4 ml. The desired concentrations of ORAs were obtained by addition to the Krebs solution in the reservoir before distension of the colon. Drug remained in the colon for 12 min during which three 40-mmHg distensions at 4-min intervals were given, followed by intracolonic instillation of the next, higher concentration of ORA. Concentration-response relationships for fentanyl and SNC-80 were obtained by intracolonic perfusion, sequentially, of 10–5, 5 × 10–5, 10–4 M or 10–4, 5 × 10–4 and 10–3 M drug concentration. EMD 71,753 and U62,066 were given in concentrations of 10–4, 5 × 10–4 and 10–3 M. At the greatest concentration of κ-ORAs tested (10–3 M), ~2.5 mg of EMD 61,753 or 1.9 mg of U62,066 were given intracolically in the volume required to distend the colon to 40 mmHg. Intra-arterial cumulative doses of EMD 61,753 and U62,066 were 0.5, 0.5, 1, 2, 4, 8, and 16 mg/kg (total cumulative dose, 32 mg/kg).

To determine whether the effects of U62,066 were produced at opioid receptors, the effects of U62,066 were tested before and 12–15 min after intracolonic administration of a nonreceptor-selective concentration of naloxone (10–5 M).

EFFECTS OF ORAS ON NOXIOUS HEAT STIMULATION. The effects of fentanyl and SNC-80, each at a dosage of 300 μg/kg intra-arterially, were tested on responses to intracolonic perfusion of heated Krebs solution. The effects of κ-ORAs on responses to heat were tested on 15 fibers, four after intracolonic administration of 10–4 M EMD 61,753 (n = 3) or U62,066 (n = 1), and 11 after intra-arterial administration of a total 16 mg/kg of EMD 61,753 (n = 3) or U62,066 (n = 8).

Data analysis

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

To estimate the response threshold to thermal stimulation, the mean and standard deviation (SD) of the resting activity was determined. Threshold was defined as the temperature at which unit activity increased >2 SD above resting activity. For fibers with no or low background activity, the response threshold was considered that temperature at which the fiber began and continued to discharge. Unit activity during thermal stimulation was counted in 10-s bins, and the maximum response during thermal stimulation was defined as that bin with the greatest number of counts.

All data are expressed as means ± SE. Results were analyzed using Student’s t-test or an ANOVA for repeated measures. The inhibitory dose 50 (ID50; dose to produce 50% inhibition of the response to distension) and 95% confidence intervals were calculated from the 20–80% component of the cumulative dose-response curve (Tallarida and Murray 1991). A value of P < 0.05 was considered statistically significant.

Drugs

Krebs solution of the following composition (in mM) 118.0 NaCl, 0.7 KCl, 24.0 NaHCO3, 1.2 MgSO4, 2.5 CaCl2, 1.1 KH2PO4, and 10.0 glucose, pH 7.3–7.4, was prepared from chemicals purchased from...
Sigma Chemical (St. Louis, MO), naloxone hydrochloride (MW: 363.8, Sigma), and fentanyl citrate (MW: 528, Abbott Laboratories, North Chicago, IL) were dissolved in saline. U62,066 (MW: 356.5, Research Biochemicals, Natick, MA) was dissolved in 10% methanol. SNC-80 (MW: 449.6, Tocris Cookson, St. Louis, MO) and EMD 61,753 (MW: 469.1, a gift from Dr. Andrew Barber, E. Merck, Darmstadt, Germany) was dissolved in 10% DMSO.

RESULTS
Fiber sample
A total of 70 mechanosensitive afferent fibers in the S1 dorsal root were studied: 24 (34%) were myelinated Aδ-fibers (mean conduction velocity: 4.9 ± 0.6 m/s, mean ± SE), and 46 (66%) were unmyelinated C-fibers (mean conduction velocity: 1.7 ± 0.1 m/s). Forty-one of 54 (76%) mechanosensitive fibers tested also responded to noxious heat; 27 were C-fibers and 14 were myelinated Aδ-fibers. Sixty-three of the total 70 fibers had an ongoing discharge (mean: 0.8 ± 0.2 imp/s; range: 0.01–6.9 imp/s); 7 fibers had no resting activity. There was no significant difference (P > 0.05) between the resting activities of C- (mean: 0.8 ± 0.2 imp/s; n = 46) and Aδ- (mean: 0.5 ± 0.2 imp/s; n = 24) fibers. All fibers gave monotonic increases in response to increasing pressures of CRD. Extrapolation of the linear portion of SRFs of these fibers revealed two populations of fibers: a large group of fibers had low thresholds (LT) for response (mean: 2.2 ± 0.4 mmHg; n = 67), and a smaller group of fibers had high thresholds (HT) for response (mean: 28.4 ± 1.3 mmHg; n = 3). SRFs of individual fibers are plotted in Fig. 1; the insets illustrate the mean SRFs of each group of fibers. The characteristics of this sample of S1 pelvic nerve afferent fibers are similar to what we found in an earlier study (Su and Gebhart 1998b).

Effects of intracolonic ORAs on responses to noxious CRD
We previously reported that responses to repetitive CRD at 40 mmHg were reproducible (Su and Gebhart 1998b) and in the present study tested responses to repetitive CRD after intracolonic administration of drug vehicle (methanol or DMSO). None of the fibers exhibited any change in response magnitude or pattern to repeated distension at a 4-min interval between distensions. Figure 2 shows the response of one fiber to 10 successive colonic distensions and the responses of each of 4 fibers to repeated CRD (see also Fig. 4 in which responses of 21 fibers do not change with repeated distension).

μ- AND δ-ORAs. The effects of intracolonic administration of fentanyl, a high efficacy μ-opioid receptor-selective agonist, and SNC-80, a δ-opioid receptor-selective agonist, were tested on the responses to noxious CRD (40 mmHg, 30 s). Fentanyl, but not SNC-80, increased the resting activity of 7/11 fibers (see Fig. 3 for example) from a mean 0.9 ± 0.4 imp/s to 2.0 ± 0.5 imp/s (10⁻³ M), 2.6 ± 0.5 imp/s (5 × 10⁻³ M) and 2.3 ± 0.5 imp/s (10⁻² M), respectively (P < 0.05). Neither fentanyl (n = 11) nor SNC-80 (n = 10), however, altered responses to noxious CRD. Figure 3 illustrates the absence of effects of these drugs on responses of different fibers. The data are summarized in Fig. 4. Responses to CRD of five fibers (3 after SNC-80 and 2 after fentanyl) were subsequently examined and

![Fig. 1](image1.png)

**FIG. 1.** Stimulus-response functions (SRFs) of 70 pelvic nerve afferent fibers to graded intensities of colorectal distension (CRD; 5–60 mmHg). *Left:* individual SRFs of 67 low-threshold afferent fibers. *Inset:* mean SRF; the mean extrapolated response threshold was 2.2 ± 0.4 mmHg. *Right:* individual SRFs of 3 afferent fibers that responded at a high threshold to CRD. *Inset:* mean SRF; the mean extrapolated response threshold was 28.4 ± 1.3 mmHg.

![Fig. 2](image2.png)

**FIG. 2.** Reproducibility of responses to repeated CRD (40 mmHg for 30 s). *A:* example of responses of a myelinated fiber (7 m/s) to 10 repeated distensions applied every 4 min. Responses of the fiber are illustrated as peristimulus time histograms (1-s binwidth); phasic distending pressure is presented below. *B:* summary of responses of 4 fibers to repeated CRD plotted as the mean increase in impulses/s over the resting activity against the number of trials. “A” indicates the example illustrated in *A*.

![Fig. 3](image3.png)

**FIG. 3.** Examples of the absence of effect of intracolonic administration of the μ-opioid receptor agonist (ORA) fentanyl and the δ-ORA SNC-80 on responses of 2 low-threshold pelvic nerve afferent fibers to CRD (40 mmHg, 30 s every 4 min). Responses are illustrated as peristimulus time histograms (1-s binwidth). Drugs were administered in concentrations as indicated. Distension pressure is presented at bottom.
inhibited after intracolonic administration of EMD 61,753 (see κ-ORA).

κ-ORA: Intracolonic administration of EMD 61,753 did not change resting activity, but concentration- and time-dependently inhibited responses to noxious CRD in 5/12 afferent fibers tested; EMD 61,753 was ineffective in 7/12 fibers. Six of 7 fibers unaffected by intracolonic administration of EMD 61,753 were then tested after intra-arterial injection of EMD 61,753; responses to CRD were dose-dependently inhibited by EMD 61,753 in all six fibers. Examples are given in Fig. 5A; data are summarized in Fig. 6. The mean maximum inhibition by EMD 61,753 given by the intracolonic route was to 49.3 ± 5.9% (n = 5) of control. The mean dose that inhibited responses to CRD to 50% of control (ID₅₀) by the intra-arterial route of administration was 6.5 ± 0.002 mg/kg. Compared with a previous study of the effects of EMD 61,753 given systemically (Sengupta et al. 1999), there was no difference between the slopes of the EMD 61,753 dose-response functions (−3.1 ± 0.6 vs. −2.1 ± 0.4), despite the difference in the method (fluid vs. air) and intensity (40 and 80 mmHg) of colonic distension.

Intracolonic administration of another κ-ORA, U62,066, also did not change resting activity, but concentration- and time-dependently inhibited responses to noxious CRD in 8/11 afferent fibers tested (Fig. 5B); U62,066 was ineffective in 3/11 fibers. The three insensitive fibers were also tested following the intra-arterial administration of U62,066, and all were dose-dependently inhibited. The data are summarized in Fig. 6. The mean maximum inhibition by U62,066 given by the intracolonic route was to 38.7 ± 8.8% (n = 8) of control. The mean ID₅₀ by the intra-arterial route was 3.5 ± 0.02 mg/kg. Compared with a previous study of the effects of U62,066 given systemically (Su et al. 1997b), there was no difference in the
slopes of the U62,066 dose-response functions (−2.7 ± 0.6 vs. −2.8 ± 0.4), despite the difference in the method and intensity of colonic distension.

To test whether the effect of U62,066 was opioid receptor mediated, the effect of intracolonic administration of U62,066 on responses of three mechanosensitive afferent fibers to noxious CRD was tested before and after intracolonic administration of naloxone (10⁻³ M). Inhibition of responses by U62,066 (10⁻³ M) before and after administration of naloxone was to 43.7 ± 10.4% and 91.2 ± 5.1% of control, respectively (P < 0.05). In two other experiments, naloxone (10⁻³ M) was instilled into the colon, and U62,066 was given in cumulative doses intra-arterially. Naloxone had no effect on control responses to 40 mmHg distension and also did not antagonize the inhibitory effects of U62,066 given intra-arterially.

Effects of κ-ORAs on intracolonic pressure

The two κ-ORAs tested here did not significantly alter colonic compliance. Intracolonic pressures during 40 mmHg CRD were changed a mean +1.2 ± 0.8, −0.3 ± 1.4 and −1.3 ± 1.4 mmHg after intracolonic administration of 10⁻⁴, 5 × 10⁻⁴ and 10⁻³ M of EMD 61,753, respectively (n = 3). Similarly, in five rats, mean intracolonic pressures changed after U62,066 (10⁻⁴, 5 × 10⁻⁴ and 10⁻³ M) a mean −1.1 ± 0.6, −3 ± 1.7 and −2.8 ± 1.8 mmHg, respectively.

Effects of ORAs on responses to noxious heat

We established in previous experiments (Su and Gebhart 1998b) that two successive heat stimuli as used here (10-min interval) did not alter mean response threshold (45.0 ± 1.4°C vs. 45.4 ± 1.3°C), mean resting activity (1.4 ± 0.5 imp/s vs. 2.5 ± 0.8 imp/s), or mean response magnitude (maximum response magnitude was 106.9 ± 18.5% of 1st heat trial).

Intra-arterial administration of the μ-ORA fentanyl (300 μg/kg) did not change the response threshold (44.6 ± 0.8 vs. 40.8 ± 0.7°C), resting activity (0.2 ± 0.1 vs. 2.8 ± 1.3), or the maximum response to heat (138.7 ± 21.6% of 1st trial) in six of seven heat-sensitive fibers (Fig. 7A). One fiber did not respond to heat after administration of fentanyl, but exhibited an afterdischarge when intracolonic temperature returned to control (37°C). In seven other heat-sensitive fibers, the δ-ORA SNC-80 (300 μg/kg) did not change either response threshold (44.8 ± 0.4 vs. 42.0 ± 1.1°C), resting activity (0.2 ± 0.1 vs. 0.8 ± 0.2), or the maximum response to heat (139.7 ± 33.5% of 1st trial).

In contrast, the κ-ORA EMD 61,753 (n = 3, intra-arterial 16 mg/kg; n = 3, intracolonic, 10⁻³ M) totally blocked responses to heat in four fibers tested and attenuated response in the other two fibers (1 intra-arterial and 1 intracolonic) to 73 and 9% of control. Response thresholds of these two fibers were not affected by EMD 61,753 (45 and 46.5°C before and 47.5 and 46°C after EMD 61,753, respectively). Similarly, U62,066 (n = 8, intra-arterial 16 mg/kg; n = 1, intracolonic 10⁻³ M) completely blocked responses to heat in eight fibers, having no effect in one fiber after intra-arterial administration. Respective examples are shown in Fig. 7, which also illustrates that responses to heat can recover 20 min after U62,066 administration.

In seven of these fibers, the effects of EMD 61,753 or U62,066 were tested on responses of pelvic nerve afferents to both mechanical and heat stimuli (intracolonic administration of EMD 61,753, n = 3; intra-arterial administration of EMD 61,753, n = 3; intra-arterial administration of U62,066, n = 1). EMD 61,753 or U62,066 attenuated the responses of pelvic nerve afferent fibers to both mechanical and heat stimuli in all seven fibers tested.

The effects of intra-arterial administration of U62,066 on responses of three mechanosensitive afferent fibers to noxious heat were tested before and 10 min after intra-arterial administration of naloxone (2 mg/kg). U62,066 (16 mg/kg) inhibited responses to heat in all three fibers. After naloxone treatment, the response to heat by U62,066 was 106.2 ± 23.5% of control; an example is shown in Fig. 8.

DISCUSSION

We previously documented that intra-arterial administration of κ-, but neither μ- nor δ-ORAs dose-dependently attenuated response of mechanosensitive pelvic nerve afferent fibers to noxious CRD (Sengupta et al. 1996, 1999; Su et al. 1997a). Similarly, in the present study, intracolonic administration of κ-, but neither μ- nor δ-ORAs concentration-dependently attenuated response of mechanosensitive pelvic nerve afferent fibers to noxious CRD. In addition, we previously reported that...
Mechanosensitive pelvic nerve afferent fibers also responded to noxious heat stimulation (Su and Gebhart 1998b). The present study confirms the polymodal character of mechanosensitive pelvic nerve afferent fibers innervating the rat colon; 78% of mechanosensitive fibers tested were also heat sensitive. Like responses to CRD, responses to noxious heat were attenuated by κ-, but neither μ- nor δ-ORAs. Drug administration in these experiments was principally by the intra-arterial route, but intracolic administration of EMD 61,753 similarly attenuated responses to intracolic perfusion of heated Krebs solution. Naloxone antagonized the effects of the κ-ORA U62,066 on both noxious mechanical and heat stimuli, providing evidence that the effects observed were produced at an opioid-like receptor, consistent with our previous reports (Sengupta et al. 1996, 1999; Su et al. 1997a,b). In those experiments where naloxone was administered intracolicly before U62,066, we noted no change in responses to noxious CRD, suggesting that no opioid tone was present peripherally or contributed to outcomes.

Intracolic κ-ORA Effects on Mechanonociception

These results extend our previous findings and establish a peripheral (colonic) site of action for κ-ORAs (in addition to or distinct from the cell soma in the DRG). Systemic administration of κ-ORAs in previous work and in the present study uniformly inhibited responses to CRD (Sengupta et al. 1996, 1999; Su et al. 1997a). Intracolic administration of κ-ORAs attenuated responses to fluid CRD of only 13 of the 23 fibers tested. This may reflect the location of some fiber terminals relatively more removed from the mucosal surface of the colon, where drugs may not have penetrated. For nine fibers unaffected by intracolic administration of κ-ORAs, intraarterial administration of κ-ORAs dose-dependently attenuated responses to noxious CRD. The magnitude of inhibition of responses to CRD differed between the different routes of drug administration, supporting the notion that there are two (or more) sites of action. In this and previous studies (Sengupta et al. 1996; Su et al. 1997a), intra-arterial administration of κ-ORAs typically inhibited responses to ≤10% of control (e.g., see Fig. 5B); the doses of EMD 61,753 and U62,066 in the present study that produced 50% attenuation of the response to CRD were 6.5 and 3.5 mg/kg, respectively. Intracolic administration of κ-ORAs attenuated responses to CRD to ~40–50% of control, which is not inconsistent with the maximum systemic concentration of drug estimated to result from intracolic instillation of 10^{-3} M EMD 61,753 or U62,066 if all drug entered the circulation (i.e., 5.06 and 3.86 mg/kg, respectively).

Although some drug administered into the colon likely entered the systemic circulation, only if we assume that all or most of the drug did so (see above), and did so over a relatively short period of time (effects of κ-ORAs were apparent when tested 6 min after intracolic administration of the 1st concentration tested, 10^{-4} M; see Fig. 5A), can the present results be interpreted to involve other than a local, colonic site of action. This interpretation is supported by the antagonistic efficacy of naloxone when both naloxone and U62,066 were given intracolicly and the failure of intracolic naloxone to antagonize intra-arterial U62,066. That responses of fibers unaffected by intracolic administration of κ-ORAs were significantly attenuated when the same drugs were given intraarterially (suggesting that drug given intracolicly does not escape in sufficient concentration into the systemic circulation) also supports a peripheral site of action of κ-ORAs.

The inhibitory effects of the κ-ORAs produced after intracolic administration also are not due to a change in compliance of the colon; intracolic pressure was not changed by the maximum concentration of κ-ORAs administered into the colon. This is consistent with a previous finding that another κ-ORA, U50,488, did not produce a significant change in tension of either longitudinal or circular muscle of the colon (Su et al. 1997a). We also failed to observe significant effects of κ-ORAs on the tone or contractility of urinary bladder smooth muscle (Su et al. 1997b). In complementary studies, κ-ORAs have been reported to not affect gastrointestinal transit in rats (La Regina et al. 1988; Tavani et al. 1984).

μ-, δ-, and κ-opioid receptors or their messenger RNAs have been documented to be present in spinal DRG cells in the rat, preferentially small DRG cells (Ji et al. 1995; Minami et al. 1995; Schafer et al. 1994). Since they were first discovered, it was appreciated that opioid receptors were present in the intestine (Pert and Snyder 1973). Pharmacological and electrophysiological investigations provided early evidence for opioid effects localized to the gastrointestinal tract, and binding studies subsequently localized opioid receptors to nerves and smooth muscle in the gastrointestinal tract (Daniel and Fox-Threlkeld 1989; Kuemmerle et al. 1992; Miller and Kirning 1989). Recent studies using antibodies raised to the cloned μ- and κ-receptors found that smooth muscle cells in the rat colon contained neither μ- nor κ-receptors (Bagnol et al. 1997; Fickel et al. 1997). Both receptor types, however, were present on myenteric and submucosal plexus neurons as well as on interstitial cells, suggesting possible roles related to absorption, secretion, motility, and visceral sensation. Resolution of which opioid receptors associated with different cells relate to which of the many effects of opioids in the colon requires further investigation. We believe that the inhibitory effects of κ-opioid receptor agonists reported here occur in consequence of activation of opioid-like receptors associated with the receptive
endings of pelvic nerve afferent fibers and/or neurons of the enteric nervous system. Finally, a recent study (Simonin et al. 1998) in κ-opioid receptor-deficient mice documented a significantly enhanced sensitivity to intraperitoneal injection of acetic acid, a model of chemical visceral nociception. In the aggregate, the present and other results demonstrate that the antinociceptive effect of κ-ORAs occurs at a peripheral receptor likely associated with afferent nerves innervating the colon. In support, Diop et al. (1996) reported in an abstract that a colonic intramural injection of the κ-OR A U50,488 (100 μg) significantly attenuated the stretching and contractions produced by a colonic intramural injection of Formalin (5%, 50 μl) in the rat.

κ-ORA effects on colonic thermonociception

With the use of cutaneous models of nociception, it was initially reported that κ-ORAs were more effective against mechanical (pressure) stimuli than noxious thermal stimulation (Millan 1989, 1990; Tyers 1980). When mechanical and thermal stimulus intensities are matched to produce equivalent response magnitudes, however, Parsons and Headley (1989a,b) found no difference in the ability of κ-ORAs to attenuate responses to these different modalities of stimulation. In the present study, only κ-ORAs and not either a μ- or δ-ORA attenuated responses to noxious visceral mechanical and thermal stimuli. Because thermal stimulation of the colon repeated more than twice, even at a 10- to 20-min interstimulus interval, sensitizes subsequent responses of pelvic nerve afferent fibers (Su and Gebhart, unpublished observations), we could not perform a quantitative, dose-dependent study of κ-ORA effects on colonic thermonociception. Comparing responses to thermal stimulation after drug with its predrug control response, we nevertheless obtained clear evidence for significant κ-ORA attenuation in response magnitude, whether given intra-arterially or intracolonically.

Like joints (Schmidt 1996), skeletal muscle (Kumazawa and Mizumura 1976; Mense 1996), dura (Bove and Moskowitz 1997), cornea (Gallar et al. 1993), splanchnic nerve afferent fibers innervating the mesentery (Adelson et al. 1996, 1997), and superior spermatic nerve innervation of the testis and/or epididymus (Kumazawa and Mizumura 1980a,b; Kumazawa et al. 1987), mechanosensitive colonic afferent fibers also transduce noxious thermal energy (Su and Gebhart, unpublished; and this study). In support, in whole cell patch-clamp recordings from adult colon sensory neurons, we (Su et al. 1999) found that nearly one-half of the DRG cells studied were capsaicin sensitive, consistent with their polymodal character (see Bevan and Szolcsányi 1990) and with documentation that the recently cloned vanilloid receptor at which capsaicin acts is a heat-transducing channel (Caterina et al. 1997).

Opioids are fairly selective in their ability to attenuate noxious inputs and have no or modest effects on other sensory modalities. Detailed information about the association of opioid receptors with polymodal nociceptors, however, is still lacking. Polymodal nociceptor axons have been described as terminating in a “chain of beads” or as having multiple sensor sites; some of these are able to be activated by mechanical stimuli, and others are depolarized by noxious heat or chemical stimuli (Szolcsányi 1993). Functionally, κ-ORAs inhibit responses of visceral polymodal afferent fibers to mechanical and thermal stimuli, and the pharmacology of modulation of heat and pressure stimuli by κ-ORAs was indistinguishable in the present study.

Implications

Intracolonic administration of κ-ORAs could provide a useful route of administration for reduction of visceral pain, reducing drug access to the CNS where κ-ORAs produce undesirable effects. In acutely irritated (HAc-, xylene- or mustard oil–treated colon or bladder) (Sengupta et al. 1996; Su et al. 1997a,b) or chronically inflamed colon (Sengupta et al. 1999), neither μ- nor δ-ORAs attenuated responses to distension, whereas the inhibitory effects of κ-ORAs were significantly greater in inflamed colon (Langlois et al. 1994; Sengupta et al. 1999). This outcome is consistent with other reports that opioids are more effective at peripheral opioid receptors in the presence of inflammation (Ferreira and Nakamura 1979; Stein et al. 1988, 1989). Accordingly, intracolonic administration of κ-ORAs may be an effective analgesic in visceral pain states such as irritable bowel disease.