Effects of Opioids on Mechanosensitive Pelvic Nerve Afferent Fibers Innervating the Urinary Bladder of the Rat

X. SU, J. N. SENGUPTA, AND G. F. GEBHART

The University of Iowa, College of Medicine, Department of Pharmacology, Bowen Science Building, Iowa City, Iowa 52242

Su, X., J. N. Sengupta, and G. F. Gebhart. Effects of opioids on ported to be mechanically insensitive (i.e., so-called silent nociceptors) and involved in nociception under pathophysiological conditions (Häbler et al. 1990). It also has been reported that most pelvic nerve afferent fibers (both myelinated and unmyelinated) in the cat are not spontaneously active when the bladder is empty. In the rat, however, pelvic nerve afferent fibers arising from the urinary bladder appear to be different from those studied in the cat; both myelinated and unmyelinated afferent fibers in the rat respond to urinary bladder distension (UBD), and the majority of the fibers are spontaneously active when the bladder is empty (Dmitrieva and McMahon 1996; Sengupta and Gebhart 1994b; Wen and Morrison 1995). All three studies in the rat documented the existence of pelvic nerve afferent fibers having either low or high thresholds for response to UBD.

Anatomic studies in the rat reveal that pelvic nerve afferent fibers innervating the urinary bladder enter the spinal cord mainly via the L6 dorsal root with a smaller fraction entering in S1 (Vera and Nadelhaft 1990, 1992). Sengupta and Gebhart (1994b) recently studied the mechanosensitive properties of pelvic nerve afferent fibers in S1 that innervated to noxious UBD (80 mmHg). Cumulative intraarterial doses of -opioid receptor agonists (morphine, 8 mg/kg, and fentanyl, 300 µg/kg) and of -opioid receptor agonists (DPDPE, 300 µg/kg, and SNC-80, 300 µg/kg) did not affect responses to noxious UBD. In contrast, cumulative 16 mg/kg intraarterial doses of the -opioid receptor agonist U50,488H, U69,593 and U62,066 dose-dependently attenuated responses to noxious UBD. There were no differences in the dose-response relationships of these drugs on afferent fibers from untreated and xylene- or mustard oil-treated urinary bladder. These results reveal that there is a greater proportion of UBD-sensitive fibers in the L6 dorsal root (57%) than in the S1 dorsal root of the rat (38%; a previous study). The attenuation of responses to UBD by , but not or -opioid receptor agonists suggests a potential use for peripherally acting -opioid receptor agonists in the control of urinary bladder pain.

INTRODUCTION

Sensations from the urinary bladder are conveyed primarily by hypogastric and pelvic nerve afferent fibers. Recent studies have led to consideration of pelvic nerve afferent fibers as important to pelvic visceral nociception (Häbler et al. 1990; Maggi 1993). In the cat, it has been proposed that myelinated pelvic nerve afferent fibers arising from the urinary bladder are mechanosensitive and are involved in nonpainful reflexes such as the micturition reflex, whereas 98% of unmyelinated pelvic nerve afferent fibers are reported to be mechanically insensitive (i.e., so-called silent nociceptors) and involved in nociception under pathophysiological conditions (Häbler et al. 1990). It also has been reported that most pelvic nerve afferent fibers (both myelinated and unmyelinated) in the cat are not spontaneously active when the bladder is empty. In the rat, however, pelvic nerve afferent fibers arising from the urinary bladder appear to be different from those studied in the cat; both myelinated and unmyelinated afferent fibers in the rat respond to urinary bladder distension (UBD), and the majority of the fibers are spontaneously active when the bladder is empty (Dmitrieva and McMahon 1996; Sengupta and Gebhart 1994b; Wen and Morrison 1995). All three studies in the rat documented the existence of pelvic nerve afferent fibers having either low or high thresholds for response to UBD.

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Visceral hyperalgesia associated with acute or chronic inflammation (e.g., interstitial cystitis) partly is due to an increase in sensitivity of the primary afferent fibers to mechanical stimulation (peripheral sensitization). For example, in the cat, production of cystitis by instilling irritants (mustard oil or turpentine oil) activated pelvic nerve afferent fibers at short latency (Häbler et al. 1990, 1993a,b). All fibers that were not previously active developed ongoing activity after instillation of irritant. In addition, afferent fibers initially insensitive to noxious distension became sensitive to nonpainful distension after the bladder was exposed to either irritant. In the rat, where mechanosensitive pelvic nerve afferent fibers are characteristically different from that of the cat, information concerning their response characteristics after acute urinary bladder irritation is lacking. The present study also examined the response properties of mechanosensitive bladder afferent fibers in the rat after acute inflammation.

The antinociceptive effects of opioids are mediated through the activation of opioid receptors in central and
peripheral tissues. To date, three opioid receptor types (µ, δ and κ) have been shown to be involved in the modulation of visceral nociception. In the spinal cord of the rat, intrathecal administration of µ- and δ-, but not κ-opioid receptor agonists significantly attenuates visceral nociception produced by noxious colorectal distension (Danzebrink et al. 1995; Harada et al. 1995a,b). Systemic administration of the κ-opioid receptor agonist U50,488, however, does attenuate responses to noxious colorectal distension. In a recent report (Sengupta et al. 1996a), we documented that systemic administration of κ-opioid receptor agonists, but not µ- or δ-opioid receptor agonists, attenuate responses of mechanosensitive afferent fibers innervating the colon of the rat. In the present report, we studied the effects of opioid receptor agonists on responses of pelvic nerve afferent fibers to UBD and found that κ-opioid receptor agonists dose-dependently attenuate responses of mechanosensitive bladder afferent fibers.

The objectives of the present study were threefold: characterize the mechanosensitive properties of pelvic nerve bladder afferent fibers in the L6 dorsal root of the rat, evaluate the effect of acute inflammation of the urinary bladder on mechanosensitive afferent fibers, and examine the effects of systemic administration of receptor-selective opioid agonists on mechanosensitive pelvic nerve afferent fibers. Some of these data have been presented in abstract form (Sengupta et al. 1995).

METHODS

General procedure

Forty-five male Sprague-Dawley rats weighing 410–530 g (Harlan, Indianapolis, IN) were used for this study. Food, but not water, was withheld for 24 h before experimentation. Rats were anesthetized initially with sodium pentobarbital (Nembutal) at a dose of 40–45 mg/kg ip and maintained thereafter with supplemental intravenous doses of pentobarbital (5–10 mg kg⁻¹ h⁻¹). The trachea was cannulated to provide artificial ventilation with room air. For injecting drugs, a catheter was passed to the descending aorta via the left common carotid artery. The femoral artery and vein were catheterized for measurement of arterial pressure and administration of pentobarbital, respectively. The rat was paralyzed with pancuronium bromide (0.3 mg/kg, iv) and subsequently ventilated with room air with a positive pressure pump (55–60 strokes/min and 3–4 ml stroke volume). Supplemental doses of pancuronium bromide (0.2–0.3 mg·kg⁻¹·h⁻¹) were given to maintain paralysis during the course of the experiment. Mean arterial blood pressure was monitored continuously and was maintained ≥80 mmHg with supplemental intravenous injection of 5% dextrose in saline solution given in 1- to 1.5-ml boluses as required. Core body temperature was maintained at 36°C by a hot-water-circulating heating pad underneath the rat and a feedback-controlled heat lamp (a thermoprobe was 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 procedure

The lower abdomen was exposed by a 3- to 4-cm-long incision laterally at the left flank. The urinary bladder was emptied and catheterized (PE-100) through the fundus. The urethra was ligated close to its entry to the penis and urine was evacuated constantly via the fundic catheter. For UBD, the bladder catheter was connected to a Mariott bottle filled with saline at room temperature. The bladder was distended with saline by regulating the air inflow into the bottle from a distension control device.

In these experiments, the descending colon and rectum also was distended to estimate the proportion of pelvic nerve afferent fibers in the left L6 dorsal root innervating the colon. For distension of the colon, a 6- to 7-cm-long, 2- to 3-cm-diam flaccid, flexible latex balloon was inserted intraanally into the descending colon and rectum. The outside diameter of the balloon when inflated was greater than the intra luminal diameter of the colon of the rat. Therefore, the pressure measured during distension indicated actual intra colonic pressure. The balloon catheter was connected to a distension control device via a low-volume pressure transducer, and the colon was distended with air (Anderson et al. 1987; Gebhart and Sengupta 1995).

The left testis, vas deferens, and seminal vesicle were tied and removed. The prostate lobe was reflected laterally to access the major pelvic ganglion and pelvic nerve. The pelvic nerve was isolated from the surrounding fatty tissues, and a pair of Teflon-coated stainless steel wires stripped at the tips were wrapped around the pelvic nerve and sealed with nonreactive silicone gel (Wacker Silicone Corp., Adrian, MI). The hypogastric, pudendal, and femoral nerves were isolated and transected. The sciatic nerve was approached through the ischiatric notch and transected.

The lumbarosacral spinal cord was exposed by laminectomy (T13–S1) and the rat was suspended from thoracic vertebra and ischia with spinal clamps. The dorsal skin was reflected lateral and tied to make a pool for mineral oil. The dura membrane was removed carefully, and the spinal cord was covered with warm (37°C) mineral oil.

Recording of afferent activity

The left L6 dorsal root was identified and decentralized at its entry to the spinal cord. Recordings were made from the distal cut end of the central processes of dorsal root fibers. A length of nerve fiber was draped over a black microbase plate immersed in warm mineral oil. The dorsal root then was split into thin bundles, and a fine filament was teased from the bundle to obtain a single unit. Electrical activity of the single unit was recorded monopolarly 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 analog delay and displayed on a storage oscilloscope after initial amplification through a low-noise AC differential amplifier. Action potentials were processed through a window discriminator and counted (1-s bin width) on-line using the spike2/CEID 1401 program. Peri-stimulus time histograms, urinary bladder or colonic distending pressures, and blood pressure were displayed on-line.

Experimental protocol

Pelvic nerve input to the L6 dorsal root was identified first by electrical stimulation of the pelvic nerve (1 0.5-ms square wave pulse at 3–8 mA). Fibers were classified on the basis of their conduction velocities; those with conduction velocities <2.5 m/s were considered unmyelinated C fibers, and those with conduction velocities >2.5 m/s were considered thinly myelinated A-fibers. The organ innervated was identified by response to brief phasic UBD (80 mmHg, 2–3 s) or CRD (80 mmHg, 2–3 s) or mechanical probing of the anal mucosa. If a fiber responded to UBD, a stimulus-response function (SRF) to phasic isometric distending pressures...
of 5, 10, 20, 30, 40, 60, 80, and 100 mmHg, 30 s each at 4-min intervals, was determined. In nine experiments, afferent fibers were isolated from the contralateral (right) L6 dorsal root. Because there was no stimulating electrode on the contralateral pelvic nerve, conduction velocities of these fibers could not be determined. All fibers, however, were mechanosensitive and gave incrementing responses to graded intensities of UBD.

To produce acute cystitis, either 0.5 ml of 30% xylenes in silicone oil (n = 11) or 5% mustard oil in peanut oil (n = 4) was instilled intravesically. The irritant was removed after 15 min, and the bladder was rinsed with saline. SRFs to phasic isotonic distension were determined again 30 and 60 min after instillation of mustard oil or xylenes. At the end of an experiment, the urinary bladder from both untreated and treated rats was removed and fixed in 10% formaldehyde. After 1 wk, the tissues were transferred to cold (4°C) 30% sucrose in 0.1 M phosphate buffer solution for cryoprotection. After 24–48 h in sucrose buffer solution, coronal sections (40 μm) were mounted on gelatin-coated glass slides and stained with hematoxylin and eosin for subsequent histological examination.

The effects of μ-opioid receptor agonists (morphine and fentanyl), δ-opioid receptor agonists (DPDPE and SNC-80), and κ-opioid receptor agonists (U50,488, U69,593, and U62,066) were tested on responses of UBD-sensitive afferent fibers (80 mmHg UBD). All drugs were administered intra-arterially in a cumulative dose paradigm. Each dose of drug was given 90 s before the onset of distension. Cumulative dose-response relationships for morphine, U50,488, U69,593, and U62,066 were obtained by giving cumulative doses of 0.5, 1, 2, 4, 8, and 16 mg/kg; cumulative doses of DPDPE, SNC-80, and fentanyl were 1, 3, 10, 30, 100, and 300 μg/kg.

Results

Sample

A total of 443 pelvic nerve afferent fibers in the L6 dorsal root were identified by electrical stimulation of the pelvic nerve; 319 (72%) were myelinated Aδ fibers with a mean CV of 11.8 ± 0.5 m/s (range: 2.5–35 m/s) and 124 (28%) were unmyelinated C fibers with a mean CV of 1.9 ± 0.03 m/s (range: 1.0–2.5 m/s). Of 443 pelvic nerve fibers, 252 (56.8%) responded to noxious UBD (80 mmHg); 108 were unmyelinated C fibers (mean CV: 1.9 ± 0.03 m/s), and 144 were myelinated Aδ fibers (mean CV: 8.2 ± 0.5 m/s). Twenty-one (4.7%) fibers responded to mechanical probing of the anal mucosa; all were myelinated Aδ fibers with a mean CV of 17.8 ± 1.7 m/s. Three (0.7%) fibers responded to noxious colorectal distension (CRD); one was unmyelinated (1 m/s) and two were myelinated (2.8 and 21.9 m/s). One hundred and sixty-seven fibers (38.1%) did not respond to UBD, CRD, or to mechanical probing of the anal mucosa. Fifteen of these afferent fibers were unmyelinated C fibers (mean CV: 1.9 ± 0.07 m/s) and 152 were myelinated Aδ fibers (mean CV: 14.2 ± 0.7 m/s). The proportions of myelinated and unmyelinated afferent fibers in the L6 and S1 dorsal roots of the rat are summarized and compared in Table 1.

Responses to UBD

Of 252 fibers that responded to UBD, 40 were studied further and characterized for responses to graded intensities of UBD (5–100 mmHg). Nine additional fibers in the contralateral (right) L6 dorsal root also were studied. Forty-six of the 49 fibers exhibited an ongoing discharge (mean rate = 1.1 ± 0.2 imp/s; range = 0.02–5.6 imp/s). The mean resting activities of 21 C and 19 Aδ fibers did not differ significantly (1.2 ± 0.3 vs. 0.7 ± 0.2 imp/s, respectively). That
both C and Aδ fibers were spontaneously active and did not differ in their resting activities is consistent with previous studies of pelvic nerve afferent fibers in the rat (Sengupta and Gebhart 1994a,b).

Responses to repeated UBD (10 trials of 80 mmHg, 20 s each at 4-min intervals) were examined in three fibers (2 Aδ and 1 C). All three demonstrated reproducible responses to repeated UBD (Fig. 1), confirming an earlier report in which a larger sample was studied in the identical way (Sengupta and Gebhart 1994b). There was no change in resting activity associated with repeated UBD at this interval.

All 49 fibers exhibited monotonic increases in response to increasing pressures of UBD. Extrapolation of the linear portion of the SRFs of these fibers to graded UBD revealed two populations of fibers, a large group of fibers having low thresholds (LT) for responses (mean = 6.0 ± 0.5 mmHg; n = 38) and a smaller group of fibers having high thresholds (HT) for responses (mean = 31.1 ± 1.2 mmHg; n = 11). Among the LT group, 16 were Aδ fibers and 14 were C fibers (6 fibers were recorded in the right dorsal root). Among the smaller HT group, 3 were Aδ fibers and 7 were C fibers (3 fibers were recorded in the right dorsal root). The relative distribution of Aδ and C fibers in the LT and HT groups is similar to the distribution of Aδ and C fibers in LT and HT groups previously found in the S1 dorsal root (Sengupta and Gebhart 1994b). Figure 2 illustrates typical responses of a LT and HT afferent fiber to graded, phasic UBD. The LT fiber in the example gives a clear response at a distending pressure of 10 mmHg whereas the HT fiber gives a modest response first at 40 mmHg. SRFs of individual fibers are plotted in Fig. 3. None of the HT fibers responded to UBD before 30 mmHg and two first responded to UBD ≥ 40 mmHg. The insets of each graph illustrate the mean SRFs for each group of fibers.

As in previous studies of pelvic nerve afferent fibers recorded in the S1 dorsal root of the rat (Sengupta and Gebhart 1994a,b), the mean response magnitudes of LT fibers was greater than the mean response magnitudes of HT fibers. In the present study, we examined whether there were any differences between the response magnitudes of Aδ and C fibers in the LT and HT groups at 100 mmHg UBD. In the LT group, the mean responses for Aδ and C fibers were 34.1 ± 4.1 (n = 16) and 35.0 ± 3.2 (n = 14), respectively. In the HT group, the mean response magnitude of the C fibers (29.3 ± 5.8, n = 7) was greater than that of the Aδ fibers (13.6 ± 2.1, n = 3). The difference is not significant (P = 0.14), likely because of small group sizes and variability in response magnitude; the greatest response was contributed to by a C fiber (see individual stimulus-response functions of HT fibers in Fig. 3).

Effect of xylenes and mustard oil treatments

The mechanosensitive properties of 11 fibers (8 LT and 3 HT) were tested before and after instillation of 0.5 ml of 30% xylenes into the bladder. The mean resting activity of 9/11 fibers increased from 0.7 ± 0.5 to 2.2 ± 0.8 imp/s (P < 0.05) 30 min after xylenes instillation and remained significantly elevated (mean: 2.5 ± 1.0 imp/s) at 60 min (Fig. 4A). Six (3 LT and 3 HT) of 11 fibers exhibited sensitization of responses to graded UBD when tested 30–60 min after instillation of xylenes. Figure 4B illustrates an example of sensitization of an unmyelinated HT fiber 60 min after xylenes treatment. One unmyelinated fiber was tested after instillation of silicone oil (vehicle for xylenes) into the bladder. Neither resting activity nor responses to graded UBD changed after silicone oil treatment.

Four fibers (3 LT and 1 HT) were tested after instillation of 0.5 ml of 5% mustard oil (MO) into the bladder. Like treatment with xylenes, the mean resting activity of these fibers increased significantly (mean: 0.6 ± 0.2 to 2.4 ± 0.4 imp/s; P < 0.05) 30 min after MO treatment and remained elevated at 60 min (mean: 2.3 ± 0.7 imp/s). Of four fibers, only the HT fiber exhibited sensitization of responses to graded UBD.

The principal effect of xylenes and MO on sensitized LT and HT fibers was an increase in response magnitude to graded intensities of UBD (Fig. 4C). In addition, the four sensitized HT fibers (xylenes, n = 3 and MO, n = 1) exhibited a significant decrease in threshold for response. The mean threshold for response of these four fibers (2 Aδ and 2 C fibers) before treatment was 28.0 ± 5 mmHg, which decreased to 7.2 ± 3.1 mmHg 60 min after irritant treatment (P < 0.05).

Effects of opioids

µ-OPIOID RECEPTOR AGONISTS. The effects of cumulative doses of morphine (8 mg/kg) or fentanyl (300 µg/kg), a high-efficacy µ-opioid receptor-selective agonist, were tested on the responses to noxious UBD (80 mmHg, 30 s) of nine mechanosensitive afferent fibers (morphine: n = 5; fentanyl: n = 4). Regardless of response threshold (7 LT and 2 HT) or CV (2 Aδ and 4 C fibers), neither of these drugs altered responses to noxious UBD. Figure 5 illustrates the absence of effects of these drugs on responses of fibers from untreated normal bladders. The effects of morphine
FIG. 1. Reproducibility of responses to repeated urinary bladder distension (UBD; 80 mmHg for 30 s). A: example of responses of an unmyelinated fiber (2.19 m/s) to 10 repeated distensions applied every 4 min. Responses of fiber are illustrated as peristimulus time histograms (PSTH; 1-s binwidth); phasic distending pressure is presented below. B: summary responses of 3 fibers to repeated UBD plotted as mean increase in imp/s over resting activity against number of trials. A indicates example illustrated in A.

and fentanyl also were tested on six fibers 30 min after instillation of xylenes or MO into the bladder (5 LT and 1 HT; 4 Aδ and 2 C fibers). These drugs were also ineffective in the presence of acute bladder inflammation. The data are summarized in Fig. 7.

κ-OPIOID RECEPTOR AGONIST. Cumulative doses of the selective δ-opioid receptor agonists DPDPE (300 μg/kg) or SNC-80 (300 μg/kg) did not alter responses to noxious UBD of 11 mechanosensitive afferent fibers (DPDPE, n = 6; SNC 80, n = 5) in the absence or presence of acute inflammation. Among this sample, eight were LT and three were HT fibers; five were Aδ and two were C fibers. Examples are shown in Fig. 6, and the data are summarized in Fig. 7.

κ-OPIOID RECEPTOR AGONISTS. All three κ-opioid receptor agonists, U50,488H, U69,593, and U62,066 (cumulative dose, 16 mg/kg), dose-dependently attenuated responses of mechanosensitive afferent fibers to noxious UBD. A total of 30 fibers were studied; 26 were LT and 4 were HT, 12 were Aδ and 14 were C fibers. We found no apparent difference in drug effects on any of these subgroups, and thus the data were pooled without respect to either response threshold or CV. Representative examples are shown in Fig. 8.

The mean ID50 values in untreated bladder were 11.4 (U50,488H), 12.8 (U69,593) and 5.1 (U62,066); the mean ID50s in acutely inflamed bladder were the same as in untreated bladder (Table 2). Figure 9 summarizes the dose-response functions of these three κ-opioid receptor agonists on responses to noxious UBD of mechanosensitive bladder afferent fibers. There were no differences in the slopes of the dose-response functions, suggesting a similar mechanism of inhibitory action. The calculated slopes of the dose-response functions in untreated bladder for U50,488H, U69,593, and U62,066 were 2.7 ± 0.3, 3.0 ± 0.3, and 3.0 ± 0.2, respectively.

To evaluate whether κ-opioid receptor agonists affect nerve conduction, CVs of six fibers were measured before and after administration of 16 mg/kg of U69,593 or U62,066. The mean CVs of these six fibers before and after U69,593 (n = 5) or U62,066 (n = 1) were 8.0 ± 2.4 and 7.4 ± 2.3 m/s, respectively.

To establish that the effects of these κ-opioid receptor
agonists were opioid receptor mediated, the effect of U50,488 on responses of two mechanosensitive afferent fibers to noxious UBD (80 mmHg, 30 s) was tested before and after systemic administration of a nonreceptor selective dose of naloxone (1 mg/kg, ia). Consistent with previous results (Sengupta et al. 1996a), the inhibition of responses by U50,488 (16 mg/kg) before and after administration of naloxone treatment was to 35 versus 64% and 32.2 versus 72.3% of control, respectively (Fig. 10).

In five experiments, the effects of cumulative doses of U50,488 were tested on five fibers after rats had been pretreated with the selective κ-opioid receptor antagonist nor-BNI (20 mg/kg, sc, 48 and 24 h before the test). In none of the five fibers tested was the inhibitory effect of U50,488 different from in untreated rats (Fig. 10).

**In vitro detrusor muscle contraction**

To examine whether the inhibitory effects of κ-opioid receptor agonists could be due to a change in compliance of the viscus (i.e., contraction or relaxation of the smooth muscle), the effects of morphine (μ-agonist), SNC 80 (δ-agonist), and U50,488 (κ-agonist) were tested on isolated detrusor muscle strips in vitro. Morphine (10⁻³ M) and U50,488 (10⁻⁴ M) produced 8.3 ± 1.1% and 2.7 ± 1.2% of the maximum contraction produced by acetylcholine (10⁻³ M), respectively. SNC-80 (10⁻⁵ M) did not produce contraction of the detrusor muscle.

**DISCUSSION**

The present study quantitatively characterized the mechanosensitive properties of bladder afferent fibers in the L6 dorsal root of the rat. About 57% of the pelvic nerve afferent fibers responded to noxious distension (80 mmHg) of the urinary bladder whereas <1% of the afferent fibers responded to noxious CRD (80 mmHg). This is in contrast to the S1 dorsal root where a significantly smaller proportion (38%) of afferent fibers were responsive to UBD, and a significantly greater proportion (16–18%) of afferent fibers responded to CRD (see Table 1) (Sengupta and Gebhart 1994a,b). There were also fewer afferent fibers in the L6
FIG. 3. Stimulus-response functions (SRFs) of 49 pelvic nerve afferent fibers to graded intensities of UBD (5–100 mmHg). A: individual SRFs of 36 low-threshold afferent fibers. Inset illustrates mean SRF; mean extrapolated response threshold was 6.0 ± 0.5 mmHg. B: individual SRFs of 13 afferent fibers that responded at a high-threshold to UBD. Inset illustrates mean SRF; mean extrapolated response threshold was 31.1 ± 1.2 mmHg.

dorsal root (4.7%) than in the S1 dorsal root (16%) that responded to probing of the anal mucosa (Sengupta and Gebhart 1994b). We found that 38% (present study), 34% (Sengupta and Gebhart 1994a), and 28.5% (Sengupta and Gebhart 1994b) of pelvic nerve afferent fibers sampled did not respond to either UBD, CRD, or probing the anal mucosa. This population of afferent fibers, some of which may innervate other pelvic organs (e.g., testes, prostate), has been characterized by others as “silent nociceptors” (Häbler et al. 1990) and represents a significantly smaller percentage in the rat than in the cat pelvic nerve (Häbler et al. 1990). Dmitrieva and McMahon (1996) recently reported that 42% (5/12) of unmyelinated pelvic nerve afferents in the rat did not respond to distension of the bladder to pressures of 60 cm H₂O.

As in the S1 dorsal root, graded isotonic distension of the urinary bladder revealed two groups of fibers; a larger group of 36 fibers (73%) had low thresholds for response (≤6 mmHg) and a smaller group of 13 fibers (27%) had high thresholds (≥30 mmHg) for response. The present study also demonstrated that acute inflammation of the urinary bladder generally caused an increase in resting activity of spontaneously active fibers or produced activity in nonspontaneously active afferent fibers. An acute inflammation of the bladder by xylenes or mustard oil also sensitized responses (i.e., increased response magnitude) to UBD. Xylenes produced sensitization in 6/11 (55%) afferent fibers tested, whereas mustard oil sensitized 1/4 (25%) afferent fibers. This observation suggests that apart from sensitization of mechanically insensitive afferent fibers (Häbler et al. 1990), acute inflammation of the urinary bladder can alter the mechanosensitive properties of many pelvic nerve afferent fibers. In addition, this study documents that κ-opioid receptor agonists, but not μ- or δ-opioid receptor agonists, can dose-dependently attenuate responses to UBD of mechanosensitive afferent fibers innervating the bladder of the rat.

Characterization of mechanosensitive afferent fibers in the L6 dorsal root

In the rat, the hypogastric and pelvic nerves passing through the major pelvic ganglion innervate the urinary bladder and urethra (in addition to colon and other pelvic organs). Retrograde horseradish peroxidase transport and elec-
Kappa opioid modulation of bladder afferent fibers. In the S1 dorsal root of the rat, we recently reported that ~62% of the pelvic nerve afferent fibers innervating the urinary bladder were unmyelinated fibers with conduction velocities \( \leq 2.5 \text{ m/s} \) (Sengupta and Gebhart 1994b). The present study reveals that in the L6 dorsal root of the rat, the proportion of unmyelinated mechanosensitive bladder afferent fibers is significantly less (43%) than in the S1 dorsal root.

Almost all (94%) bladder afferent fibers in the L6 dorsal root of the rat exhibited ongoing resting activity similar to what has been observed in the S1 dorsal root, where 90% of the bladder afferent fibers had resting activity (Sengupta and Gebhart 1994b). In the L6 dorsal root, a study using antidromic activation of bladder afferent fibers in the rat pelvic nerve reported that 70% of the fibers were unmyelinated (\( \approx 2.5 \text{ m/s} \)) (Vera and Nadelhaft 1990). Electron microscopy studies have demonstrated the location and distribution of the pelvic nerve afferent fibers from the lower urinary tract and their cell bodies in the lumbosacral dorsal root ganglia (Applebaum et al. 1980; Morgan et al. 1981; Nadelhaft et al. 1983; Nadelhaft and Booth 1984; Nance et al. 1988; Pascual et al. 1989, 1993; Vera and Nadelhaft 1992). In the Sprague-Dawley rat, it is estimated that 84% of the pelvic nerve afferent fibers supplying the lower urinary tract enter the spinal cord via the L6 dorsal root (Vera and Nadelhaft 1990). A study using antidromic activation of bladder afferent fibers in the pelvic nerve reported that 70% of the fibers were unmyelinated (\( \approx 2.5 \text{ m/s} \)) (Vera and Nadelhaft 1990).
and Gebhart 1994b). This is in contrast to the cat in which it has been reported that \( \sim 90\% \) of the bladder afferent fibers in the pelvic nerve have no ongoing activity (Bahns et al. 1987; Häbler et al. 1990, 1993a,b). Recently, Dmitrieva and McMahon (1996) reported in the rat that most pelvic nerve bladder afferents had low levels of ongoing activity. Accordingly, there appear to be species differences or significant methodological differences that contribute to findings in rat and cat. Regarding methodological differences, the rate of ongoing activity in the present and a previous study (Sengupta and Gebhart 1994b) was greater than reported by Dmitrieva and McMahon (1996). Our procedures involved catheterization of the bladder through the fundus and ligating the urethra (possibly producing sensitization of some afferents) whereas Dmitrieva and McMahon (1996) cannulated the bladder transurethrally. However, in experiments in which the rat colon was not potentially sensitized by tissue damage [because the balloon is inserted via the anus (Sengupta and Gebhart 1994a)], the resting activities of \( \text{A}\delta \) and C fibers in the S1 dorsal root were the same as recorded in the L6 dorsal root in the present study. Dmitrieva and McMahon (1996) also used female rats, likely in different phases of the estrus cycle, that were anesthetized with urethane; pentobarbital-anesthetized male rats were used in the present study.

As in the S1 dorsal root (Sengupta and Gebhart 1994b), both unmyelinated and myelinated afferent fibers in the L6 dorsal root were found to be mechanosensitive to UBD. This is in contrast to the character of pelvic nerve afferent fibers innervating the bladder of the cat where almost all (98%) unmyelinated fibers were reported to be mechanically insensitive (Häbler et al. 1990). Responses of the mechanosensitive afferent fibers to graded intensities of distension of the urinary bladder of the rat revealed the existence of afferent fibers having low or high thresholds for response. The proportion of high-threshold afferent fibers (27%) was the same as in our previous study in the S1 dorsal root (Sengupta and Gebhart 1994b). The existence of high-threshold bladder afferent fibers in the L6 and S1 dorsal roots of the rat also has been reported by Wen and Morrison (1995) and Dmitrieva and McMahon (1996), consistent with the present and an earlier report (Sengupta and Gebhart 1994b), where thresholds for response of some fibers exceeded 40 mmHg. The proportion of such HT fibers reported by others, however, is less than reported here. In the preliminary report by Wen and Morrison (1995), the bladder was distended slowly to intravesical pressures in excess of 40 mmHg. Distension pressures and response thresholds were not reported, but 1/19 fibers studied was classed as HT. Similar to the present report, both \( \text{A}\delta \) and C fibers were found to have low response.

**FIG. 5.** Examples of effects of \( \mu \)-opioid-receptor agonists on responses of pelvic nerve afferent fibers to UBD (80 mmHg, 30 s every 4 min). Drugs were injected intra-arterially in a cumulative dose as indicated (†). A: responses of a LT C fiber to UBD after a cumulative 8 mg/kg dose of morphine. B: responses of a LT A\( \delta \) fiber to UBD after a cumulative 300 \( \mu \)g/kg dose of fentanyl.
FIG. 6. Examples of effects of δ-opioid receptor agonists on responses of pelvic nerve afferent fibers to UBD (80 mmHg, 30 s every 4 min). Drugs were injected intra-arterially in a cumulative dose as indicated (†). A: responses of a LT Aδ fiber to UBD after a cumulative 300 μg/kg dose of DPDPE. B: responses of a LT Aδ fiber to UBD after a cumulative 300 μg/kg dose of SNC-80.

thresholds. Among 21 mechanosensitive fibers studied by Dmitrieva and McMahon (1996), two fibers had response thresholds >40 mmHg. We have defined LT and HT on the basis of extrapolated response thresholds and the observation that there are two populations of fibers in the pelvic nerve of the rat that innervate the urinary bladder or colon (see Sengupta and Gebhart 1995). One explanation for differences in the proportion of HT fibers reported here and those found in other studies may relate to the criteria applied to define them. The mean response threshold of HT fibers in the present report was 32 mmHg, an intensity of UBD less than used by either Wen and Morrison (1995) or Dmitrieva and McMahon (1996) to apparently identify a fiber as HT.

Responses of mechanosensitive afferent fibers to irritant chemicals

Experimental inflammation of the urinary bladder has been produced by exposing the urothelium to irritant chemicals. For example, mustard oil and xylenes can produce, depending on duration of exposure and concentration of irritant, desquamation, hemorrhagic patches, polymorphonuclear (PMN) leukocyte infiltration, edema, and plasma extravasation in bladder tissue (Abelli et al. 1988, 1989, 1991; Hahler et al. 1990, 1993a,b; Koltzenburg and McMahon 1986; Maggi et al. 1988; McMahon and Abel 1987). Similar to earlier studies, in the present study, both xylenes and mustard oil caused PMN cell infiltration into bladder tissues. We also observed PMN cell infiltration in untreated bladder tissue that underwent repeated (>10 trials) noxious UBD (80 mmHg, 30 s). PMN cell infiltration due to repeated noxious distension of untreated bladder could be mediated by prostaglandins, as it has been reported that an excessive stretch of detrusor muscle can induce release of prostaglandins from the urothelium which in turn can lead to PMN cell infiltration into the tissues (Khalaf et al. 1979).

The primary cause of inflammation by mustard oil and xylenes is release of vasoactive substances (e.g., substance P, CGRP) from capsaicin-sensitive primary afferent fibers (Lundberg et al. 1984; Maggi et al. 1987, 1988; McMahon and Abel 1987). Chemical denervation of these afferent fibers by capsaicin or major pelvic ganglionectomy prevents inflammation produced by mustard oil or xylenes (Alessandri et al. 1988; Maggi et al. 1988; McMahon and Abel 1987). Xylenes, a well known C-fiber irritant (Jancso et al. 1967, 1968), may act at the level of sensory nerve endings by causing depolarization and subsequent peripheral release of stored vasoactive peptides (Maggi et al. 1987, 1988). Abelli et al. (1988) demonstrated that xylenes-induced bladder hyperreflexia and plasma extravasation were due to release of substance P and related tachykinins and CGRP. Immunohistochemical examination of bladder tissue indicated that...
these peptides co-exist in the same capsaicin-sensitive primary afferent fibers in the rat bladder (Sundler et al. 1985). Like xylenes, mustard oil has direct excitatory effects on primary afferent fibers. Häbler et al. (1990) demonstrated that in the cat S2 dorsal root, a large number of unmyelinated, mechanically insensitive afferent fibers could be activated by instillation of mustard oil or turpentine oil into the urinary bladder. In a subsequent study, these investigators demonstrated excitation and sensitization of myelinated mechanosensitive afferent fibers by the same irritants (Häbler et al. 1993a). Excitation of myelinated afferent fibers by irritant chemicals is a direct effect and not secondary to altered mechanical transduction properties or bladder hyperreflexia (Häbler et al. 1993b; McMahon and Abel 1987). In the present study, xylenes increased resting activity of 9/11 fibers (8 LT and 3 HT) tested and increased the response magnitude of 6/11 fibers. Xylenes sensitized all four HT afferent fibers tested, similar to previous observations where 2.5% acetic acid treatment of the colon sensitized all HT afferent fibers, but only 50% of LT afferent fibers (Sengupta et al. 1996). The enhanced response magnitude of mechanosensitive afferent fibers to bladder distension was observed when tested 30 min after xylenes instillation into the bladder and was unchanged after 60 min. Mustard oil similarly increased the resting activity of all fibers tested (3 LT and 1 HT) but sensitized responses to UBD of only the HT fiber.

**Effects of opioids**

Traditionally, it was believed that the analgesic effects of opioids were mediated by actions in the CNS. However, several studies in recent years have demonstrated peripheral antinociceptive effects of opioids (for reviews, see Barber and Gottschlich 1992; Millan 1990; Stein 1995). Fewer studies have examined opioid effects on primary afferent fibers. Russel et al. (1987) demonstrated in the articular nerve innervating the knee joint of the cat that μ-receptor agonists had a modest or no effect on resting activity of the fibers studied whereas κ-receptor agonists decreased resting activity of these fibers. Similarly, in an in vitro skin-tibial nerve preparation, a κ-receptor agonist (U69,593) produced a decrease in resting activity of all fibers tested (Andreev et al. 1996).

**FIG. 7.** Summary of dose-response functions after cumulative doses of μ (A: morphine and B: fentanyl) or δ (C: DPDPE and D: SNC 80)-opioid-receptor agonists on responses of pelvic nerve afferent fibers to noxious UBD (80 mmHg, 30 s). None of these drugs attenuated responses of pelvic nerve afferent fibers from untreated or xylenes-treated bladders.
FIG. 8. Examples of effects of κ-opioid-receptor agonists on responses of pelvic nerve afferent fibers to UBD (80 mmHg, 30 s every 4 min). Drugs were injected intra-arterially in a cumulative dose as indicated (†). A: responses of a LT C fiber to UBD after a cumulative 16 mg/kg dose of U50,488. B: responses of a LT Aδ fiber to UBD after a cumulative 16 mg/kg dose of U69,509. C: responses of a LT C fiber to UBD after a cumulative 16 mg/kg dose of U62,066.

Morphine attenuated resting activity in that study only after inflammation of the skin by UV exposure. In the rat, intrathecal application of μ- and δ-opioid receptor agonists, but not the κ-receptor agonist U50,488, attenuated pseudosomatic response to noxious CRD. In the same model, κ-receptor agonists produced an antinociceptive effect when injected systemically, suggesting a peripheral action (Danzebrink et al. 1995; Diop et al. 1994; Harada et al. 1995a,b). This antinociceptive effect of κ-agonists could be due to inhibition of transduction processes at the sensory endings, axon or cell body by an action at κ-opioid receptors; blocking propagation of nerve action potentials; or a change in compliance of the viscus. In the present study, conduction velocities of the fibers remained unaffected after high doses of U50,488 or U62,066 or U69,593. In addition, in vitro recording of the tension of the detrusor muscles revealed that 100 μM U50,488 did not produce a significant change in tension of the muscle. Accordingly, the effects reported here likely occur at receptors associated with afferent nerves innervating the urinary bladder.

TABLE 2. Effective dose for 50% inhibition (ID50) and 95% confidence intervals for U50,488, U62,066, and U69,593

<table>
<thead>
<tr>
<th></th>
<th>U50,488</th>
<th>U62,066</th>
<th>U69,593</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>11.4 (3.9–33.2)</td>
<td>5.1 (4.6–5.6)</td>
<td>12.8 (2.7–55.2)</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Acutely inflamed</td>
<td>8.7 (4.5–17.1)</td>
<td>14.0 (7.7–25.5)</td>
<td>17.2 (9.2–35)</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>nor-BNI pretreated (48 h)</td>
<td>8.4 (1.9–37.3)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
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et al. 1994; Takemori et al. 1988). In an ongoing examination of agonists at \(\kappa\)-opioid-receptor subtypes, we (unpublished data) have been unable to antagonize the effects of any \(\kappa\)-opioid-receptor agonist in this preparation with nor-BNI or another \(\kappa\)-opioid receptor-selective antagonist (DIPPA); naloxone has been uniformly effective. We interpret these results to suggest that the \(\kappa\)-opioid receptor in the periphery is not the same as characterized in the CNS.

An increased antinociceptive effect of systemically administered opioid receptor agonists has been reported in animals after the induction of peripheral inflammation (e.g., Joris et al. 1990; Kayser and Guilbaud 1983; Neil et al. 1986). This has been attributed to actions of these drugs at periphery opioid receptors, which become functional (de novo synthesis) and/or upregulated in inflammation (Hargreaves et al. 1988; Stein et al. 1988, 1989) and central changes (Hylde et al. 1991). Both central and peripheral changes in opioid sensitivity have been shown to occur within 2 h of the onset of the inflammation (Stanfa et al. 1992). It has been proposed that \(\mu\)-opioid-receptor agonists act to inhibit activation of adenylyl cyclase in peripheral afferent neurons by inflammatory mediators such as serotonin and prostaglandin \(E_2\), whereas \(\delta\)- and \(\kappa\)-opioid-receptor agonists also may inhibit secretion of pro-inflammatory substances by sympathetic neurons. (Levine and Taiwo 1989; Taiwo et al. 1992; Taiwo and Levine 1991). In the present study, however, neither \(\mu\)-nor \(\delta\)-opioid-receptor agonists were efficacious in attenuating responses of afferent fibers to noxious distension after urinary bladder inflammation. Moreover, although \(\kappa\)-opioid-receptor agonists dose-dependently attenuated responses of afferent fibers to noxious UBD, their potency was not increased after urinary bladder inflammation. Because drug effects were tested within 2 h of irritant instillation into the urinary bladder, it may be that the absence of effect of \(\mu\)- and \(\delta\)-receptor agonists and the failure of the dose-response functions for \(\kappa\)-receptor agonists

**FIG. 9.** Dose-dependent inhibition by \(\kappa\)-opioid-receptor agonists on responses of pelvic nerve afferent fibers to UBD. Mean inhibition of responses to UBD (as percent of control) by U50,488 (A); U69,593 (B); or U62,066 (C). There were no apparent differences in effects of these drugs in untreated or xylene-treated conditions.

In this and a previous report (Sengupta et al. 1996a), naloxone at a nonreceptor-selective dose antagonized the inhibitory effect of U50,488 on responses to noxious UBD or colorectal distension, suggesting that the effects of the \(\kappa\)-opioid receptor agonists studied were produced at opioid receptors. The \(\kappa\)-opioid receptor-selective antagonist nor-BNI, however, was ineffective regardless of the pretreatment regimen used for the antagonist (which has a long latency to onset of effect and a long duration of action) (Spanagel et al. 1994).

**FIG. 10.** Effects of naloxone (NLX; 1 mg/kg) and nor-BNI on inhibitory effect of U50,488 (8 mg/kg) on responses to noxious UBD (80 mmHg, 30 s). Nor-BNI was given in 2 doses of 20 mg/kg (48 and 24 h) before test.
to shift leftward reflects an inadequate time for de novo receptor synthesis or upregulation. We recently have studied the effects of κ-receptor agonists on pelvic nerve afferent fiber responses to noxious colonic distension 4 day after chronic inflammation of the colon and found a leftward shift in the dose-response function (Sengupta et al. 1996b). There was, however, no apparent change in the inability of morphine or SNC 80 to attenuate responses to CRD.

These results suggest that peripherally acting κ-opioid-receptor agonists could be useful in modulating the pain associated with bladder dysfunction, such as in interstitial cystitis.

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