Epinephrine Produces a β-Adrenergic Receptor-Mediated Mechanical Hyperalgesia and In Vitro Sensitization of Rat Nociceptors

SACHIA G. KHASAR, GORDON MCCARTER, AND JON D. LEVINE
Departments of Medicine and Oral and Maxillofacial Surgery, Division of Neuroscience and Biomedical Sciences Program, National Institutes of Health Pain Center (UCSF), University of California, San Francisco, California 94143-0440

INTRODUCTION

A contribution of catecholamines to both inflammatory and neuropathic pain states has been suggested based on both clinical evidence and animal models (Jänig et al. 1996; Raja 1998). Although most research in the area has evaluated the contribution of the sympathetic postganglionic neuron (SPGN) and its major transmitter norepinephrine, these pain states also may be associated with increased activity of the sympathoadrenal system, leading to elevation of the plasma concentrations of epinephrine [an endogenous β-adrenergic receptor (β-AR) agonist] (Cryer 1980; DeTurck and Vogel 1980; Taylor et al. 1989; Wortsman et al. 1984). Although activation of β-ARs was shown, almost two decades ago, to produce behavioral hyperalgesia (Ferreira 1980) and epinephrine can cause anginal pain in the absence of apparent ischemia (Eriksson et al. 1995), further studies of the role of β-ARs in peripheral pain and the elucidation of mechanisms involved have been lacking. We investigated mechanisms involved in epinephrine-induced hyperalgesia, hypothesizing that epinephrine produces hyperalgesia by directly sensitizing primary afferent nociceptors.

We tested this hypothesis in vivo using the behavioral model of mechanical hyperalgesia and in vitro using cultured dorsal root ganglion (DRG) neurons. We also tested whether epinephrine enhances tetrodotoxin-resistant sodium currents (TTX-R $I_{Na}$), which are carried by an ion channel selectively found in primary afferent nociceptors (Akopian et al. 1996; Sangameswaran et al. 1996) and the activity of which is increased by agents that act directly on primary afferent nociceptors in vivo to produce hyperalgesia (England et al. 1996; Gold et al. 1996b). Finally, we examined the second-messenger systems that may mediate the hyperalgesic and sensitizing effects of epinephrine.

METHODS

Experimental procedures

BEHAVIORAL EXPERIMENTS. Behavioral experiments were performed on lightly restrained male Sprague-Dawley rats (250–350 g) purchased from Bantin and Kingman (Fremont, CA) and housed in the animal care facility of the University of California, San Francisco, under a 12-h light/dark cycle. Care and use of rats conformed to National Institutes of Health guidelines, and experimental protocols were approved by the University of California, San Francisco, Committee on Animal Research. The nociceptive flexion reflex was quantified by an Ugo Basile Analgesimeter (Stoelting, Chicago, IL), which applies a linearly increasing mechanical force to the dorsal surface of the rat’s hind paw.

Before the rats were used for behavioral experiments, they were trained in the paw-withdrawal reflex test at 5-min intervals for 1 h each day for a period of 5 days. This training procedure reduces variability and produces a more stable baseline paw-withdrawal threshold measurement, thereby enhancing the ability to detect the effect of agents that modulate nociception (Taiwo et al. 1989). On the
day of the experiments, paw-withdrawal threshold was measured (i.e., rats were exposed to the test stimulus) at 5-min intervals for 1 h. The mean of the last six paw-withdrawal thresholds was determined. This mean value is defined as the baseline paw-withdrawal threshold before the injection of a test agent. Test agents were injected intradermally into the dorsal surface of both hindpaws, in a volume of 2.5 µl. Paw-withdrawal thresholds then were determined at 10, 15, and 20 min postinjection. The mean of the paw-withdrawal thresholds obtained at these three time points is the mechanical nociceptive threshold at the dose of the test agent used. The effect of each dose of a test agent was calculated as the percentage change from baseline, for each paw, as follows: [(threshold in presence of test agent minus baseline threshold)/baseline threshold] × 100. This transformation was done to account for the differences in baseline thresholds of individual rats within a group. Increasing doses of test agents, each an order of magnitude greater than the previous dose, were injected cumulatively at 25-min intervals. Because we have shown, using this protocol under similar experimental conditions, that repeated injection of small volumes of PGE₂ locally, in one paw, does not affect the protocol under similar experimental conditions, that repeated injection of small volumes of PGE₂ locally, in one paw, does not affect the baseline paw-withdrawal threshold also was tested. The dose-response relationship for epinephrine was determined 7 days after surgical sympathectomy or sham surgery. To test if epinephrine hyperalgesia is dependent on the presence of intact sympathetic postganglionic neurons (SPGNs), rats were sympathectomized surgically under pentobarbital anesthesia (50 mg/kg body wt, with additional doses given to maintain areflexia during surgery). After a lateral abdominal incision, the sympathetic chain was reached via an extraperitoneal approach, and the lumbar sympathetic chains, from ganglia L₁ to L₄, were removed bilaterally (Baron et al. 1988; Miao et al. 1995). This procedure results in complete sympathetic denervation of the hind limbs (Baron et al. 1988). Sham surgeries were performed in a similar manner, except that the sympathetic chains were left intact, after exposure. The dose-response relationship for epinephrine-induced hyperalgesia was determined 7 days after surgical sympathectomy or sham surgery.

SYMPATHECTOMY. To test if epinephrine hyperalgesia is dependent on the presence of intact sympathetic postganglionic neurons (SPGNs), rats were sympathectomized surgically under pentobarbital anesthesia (50 mg/kg body wt, with additional doses given to maintain areflexia during surgery). After a lateral abdominal incision, the sympathetic chain was reached via an extraperitoneal approach, and the lumbar sympathetic chains, from ganglia L₁ to L₄, were removed bilaterally (Baron et al. 1988; Miao et al. 1995). This procedure results in complete sympathetic denervation of the hind limbs (Baron et al. 1988). Sham surgeries were performed in a similar manner, except that the sympathetic chains were left intact, after exposure. The dose-response relationship for epinephrine-induced hyperalgesia was determined 7 days after surgical sympathectomy or sham surgery.

CELL CULTURE AND ELECTROPHYSIOLOGY. To examine the effect of epinephrine on the excitability and ionic conductance of isolated DRG neurons, primary cultures of adult rat lumbar DRGs (L₁–L₄) were prepared as previously described (Gold et al. 1996a). Culture medium consisted of minimal essential medium (MEM) supplemented with 10% fetal bovine serum and 1,000 units per ml each of penicillin and streptomycin. Neurons were plated onto glass cover slips coated with laminin and poly-1-ornithine and were maintained in culture medium with nerve growth factor (NGF; GIBCO BRL, Gaithesburg, MD) at 37°C under 3% CO₂. Neurons were used within 24 h of plating before there was appreciable outgrowth of neurites. Small-diameter neurons (20–30 µm in diameter) were studied because they selectively express properties of nociceptors (Gold et al. 1996a). Bath solution continuously perfused the recording chamber at 1–2 ml/min. Drugs were added as described in the respective figure legends. Experiments were performed at room temperature (21−24°C).

Whole cell patch-clamp recordings were performed using an Axopatch 200B amplifier with pClamp6 acquisition and stimulation programs (Axon Instruments, Foster City, CA). Data were low-pass filtered at 5 kHz and acquired at 20 kHz. For current-clamp recordings, the perforated-patch method was employed with borosilicate glass electrodes fire-polished to 1–3 MΩ. The electrode solution contained (in mM) 30 KCl, 55 K₂SO₄, 1 CaCl₂, 2 MgCl₂, 11 EGTA, and 10 HEPES, pH adjusted to 7.2 with KOH, and osmolarity adjusted to 310 mOsm with sucrose. Nystatin was dissolved in DMSO at 6 mg/100 µl for each day’s experiments, and 2 µl of this stock solution
was added to 300 μl of electrode solution before filling each electrode. The current-clamp bath solution contained (in mM) 130 NaCl, 3 KCl, 2.5 CaCl₂, 0.6 MgCl₂, 10 HEPES, and 10 glucose, pH adjusted to 7.4 with NaOH, and osmolarity adjusted to 325 mOsM with sucrose. A neuron was only used if its resting potential was more negative than −45 mV and could be maintained throughout the experiments at −60 mV by constant current injection. Stimulation current was injected in a ramp-and-plateau protocol in which the current was increased linearly for 250 ms and then held at the final level for an additional 500 ms. The final amplitude of the injected current was adjusted to depolarize the neuron enough so that one to three action potentials were triggered during the ramp-and-plateau stimulus.

Voltage-clamp experiments were performed with 2- to 5-MΩ electrodes filled with (in mM) 140 CsCl, 10 NaCl, 0.1 CaCl₂, 2 MgCl₂, 11 EGTA, 10 HEPES, 2 Mg-ATP, and 1 Li-ATP; pH was adjusted to 7.2 with Tris-base. Bath solution contained (in mM) 35 NaCl, 30 tetraethylammonium chloride, 65 choline chloride, 0.1 CaCl₂, 5 MgCl₂, 10 HEPES, and 10 glucose; pH was adjusted to 7.4 with NaOH, and osmolarity adjusted to 325 mOsM with sucrose. Tetrodotoxin (TTX, 50 nM) was added to the bath solution. Capacitance and series resistance (>80%) was compensated, and leak subtraction was performed with a P/4 protocol. After obtaining a current-voltage (I-V) relationship for TTX-R Is, a test pulse was applied every 20 s to monitor the amplitude of TTX-R Is during the experiment. A voltage that produced approximately half-maximal current activation was used for the test pulse because hyperalgesic agents produce the greatest increase in TTX-R Is, at this part of the I-V curve (Gold et al. 1996b) (also see Fig. 5B).

Materials

Drugs or reagents used in this study were from Sigma (St. Louis, MO) unless otherwise noted: prostaglandin E₂ (PGE₂), epinephrine [an endogenous β-adrenergic receptor (β-AR) agonist], isoproterenol (a specific β-AR agonist), propranolol (a specific β-AR antagonist), phentolamine (a specific α-adrenergic receptor (α-AR) antagonist), CIBA-GEIGY, Summit, NJ), Damgo, enkephalin (a μ-opioid receptor agonist; Research Biochemicals, Natick, MA); SQ 22536 (an adenylyl cyclase inhibitor); BIM (a PKC inhibitor) (both from Calbiochem, La Jolla, CA); chelerythrine (a PKC inhibitor; L. C. Labs., Woburn, MA); Rp-cAMPs (a PKA inhibitor; Biolog, La Jolla, CA); WIPITIDE (a PKA inhibitor; Peninsula Labs, Belmont, CA); indomethacin sodium salt (a generous gift from Merck Research Labs, Rahway, NJ). PGE₂ (4 mg/ml) stock solution was made by dissolving it in 10% ethanol in normal saline; further dilutions were made by adding saline. Final concentration of ethanol was <1%. Isoproterenol, phentolamine, propranolol, chelerythrine, BIM, and WIPITIDE were dissolved in distilled water. Epinephrine (4 mg/ml) was dissolved in distilled water with an equivalent amount of ascorbic acid just before it was used and was kept on ice in subdued lighting conditions. All other drugs were dissolved in normal saline or bath solution.

Data are presented as means ± SE and analyzed statistically using one-factor ANOVA or repeated measures ANOVA as appropriate. Where ANOVA showed significant differences between groups, Fisher’s protected least-significant difference (PLSD) post hoc test was used to determine the specific pairs of groups between which statistically significant differences occurred. P < 0.05 was the accepted level for statistical significance.

Results

Behavioral studies

Epinephrine (1 ng to 1 μg) produced a dose-dependent decrease in the mechanical nociceptive threshold (i.e., produced hyperalgesia; F = 90.7, P < 0.01; one-factor ANOVA) when injected intradermally into the dorsal surface of the hindpaw of the rat (mean baseline paw-withdrawal threshold for this group of rats was 107.5 ± 1.2 g; n = 31) (Fig. 1A). The intradermal injection of ascorbic acid (4 mg/ml) did not significantly affect basal paw-withdrawal threshold (data not shown). The latency to onset of epinephrine (1 μg)-induced hyperalgesia was brief (Fig. 2); it was significant 2 min after injection, reached peak effect by 5 min (Fig. 2A) and lasted ~2 h (Fig. 2B). Epinephrine-induced hyperalgesia was attenuated by treating the paws (mean baseline paw-withdrawal threshold 102.6 ± 2.2 g; n = 8) with propranolol, significantly shifting the dose-response curve to the right of that for epinephrine alone (Fig. 1A). Injection of propranolol alone did not alter basal paw-withdrawal threshold (data not shown). Pretreatment of paws with distilled water and subsequent coinjection with epinephrine during the course of the experiment also produced dose-dependent hyperalgesia (F = 40.76, n = 14; Fig. 1B). Repeated injections of distilled water alone did not produce hyperalgesia (F = 1.48, n = 4; Fig. 1A).

Because epinephrine has affinity for both α- and β-ARs, we evaluated the contribution of the α-AR to epinephrine-induced hyperalgesia. We tested epinephrine-induced hyperalgesia against phentolamine, an α-AR antagonist. Earlier studies (Levine et al. 1986) showed that injection of phentolamine alone had no effect on basal paw-withdrawal threshold in the normal rat. In the current study, phentolamine did not significantly affect epinephrine-induced hyperalgesia (Fig. 1A) The mean baseline paw-withdrawal threshold of this group of rats was 107.0 ± 1.8 g; n = 17. A similar dose for phentolamine was shown to significantly reverse formalin-induced decrease in paw threshold (Levine et al. 1986) and also inhibit rolipram-induced prolongation of PGE₂ hyperalgesia (Ouseph et al. 1995). All data from the groups of rats in Fig. 1. A and B, were analyzed together. ANOVA showed significant differences between the groups (F = 12.32; P < 0.01).

It has been shown that bradykinin (BK) and norepinephrine act on intermediary cells to trigger prostaglandin synthesis, which then acts on the primary afferent to sensitize it (Lembeck et al. 1976; Taiwo and Levine 1988). We therefore tested whether the inhibition of prostaglandin synthesis by indomethacin would affect epinephrine-induced hyperalgesia. Indomethacin (4 mg/kg ip, pretreatment, and 1 μg id, during the course of the experiment), did not affect the ability of epinephrine to produce hyperalgesia (Fig. 1B). The mean baseline paw-withdrawal threshold of this group of rats was 109.2 ± 6 g; n = 8. Earlier studies (Levine et al. 1986; Taiwo et al. 1990) showed that injection of indomethacin alone has no effect on basal paw-withdrawal threshold in the normal rat. Because BK and norepinephrine also act indirectly via sympathetic neurons to affect nociceptors (Levine et al. 1986), we tested the effect of sympathectomy on epinephrine-induced hyperalgesia. The elimination of sympathetic innervation of the hindpaw by sympathectomy had no effect on the hyperalgesia produced by epinephrine (Fig. 1B). The mean baseline paw-withdrawal threshold of this group of rats was 104.2 ± 3.2 g; n = 8, for the sympathectomized and 113.6 ± 3.2 g; n = 6, for the sham-sympathectomized group.

To further explore the contribution of the β-AR to hyperalgesia, we used the β-AR-selective agonist, isoproterenol (Ahlquist 1976). Intradermal injection of isoproterenol (1 ng to 1 μg), like epinephrine, produced dose-dependent hyperalgesia...
The mean baseline paw-withdrawal threshold of this group of rats was 103.1 ± 1.5 g; n = 20. Propranolol significantly attenuated the hyperalgesia produced by isoproterenol, whereas phentolamine had no effect. The mean baseline paw-withdrawal threshold of the isoproterenol + propranolol group of rats was 101.3 ± 2.7 g; n = 8 and that for the isoproterenol + phentolamine group was 111.3 ± 2 g; n = 8.

The intradermal coinjection of DAMGO (1 µg) with epinephrine (100 ng) or intradermal pretreatment of rat paws with SQ 22536, chelerythrine, BIM, Rp-cAMPS (all 1 µg), or WIPTIDE (100 ng) 15 min before injection of epinephrine, significantly attenuated epinephrine-induced hyperalgesia. The same dose of chelerythrine or BIM had no effect on PGE2-induced hyperalgesia (Table 1). BIM (1 µg) also significantly attenuated isoproterenol hyperalgesia and WIPTIDE (100 ng) almost completely abolished it (Table 1). The intradermal injection of none of these agents alone affects basal paw-
withdrawal threshold [Aley and Levine 1997, (for DAMGO) and unpublished observations].

In vitro electrophysiological studies with cultured DRG neurons

To test the hypothesis that epinephrine-induced hyperalgesia was mediated by a direct effect of epinephrine on the primary afferent nociceptor, we performed whole cell patch-clamp electrophysiological experiments on dissociated DRG neurons in culture. Small-diameter (i.e., 20–30 μm) neurons were used within 12–24 h of plating as a model for peripheral nociceptor neurons. This is based on data showing similarities in the pharmacological repertoires of the cell body in vitro after 12–24 h in culture and the primary afferent nociceptor terminal in vivo (Baccaglini and Hogan 1983; England et al. 1996; Gold et al. 1996a; Pitchford and Levine 1991).

Current-clamp recordings were performed using the perforated-patch whole cell technique. The number of action potentials generated during a 750-ms ramp-and-plateau depolarizing current injection (see METHODS), as well as the latency to the first spike, were used as measures of excitability. After 5–10 min of baseline recordings, epinephrine (1 μM) was added to the bath. Figure 4A shows voltage traces from a typical neuron before and during exposure to 1 μM epinephrine, whereas Fig. 4B shows the time course of changes in the number of action potentials and the latency to the first action potential for another cell. For 11 neurons treated with 1 μM epinephrine, the average number of action potentials generated in response to the current ramp-and-plateau stimulus was 1.7 ± 0.2 before the addition of epinephrine and 5.3 ± 0.9 5 min or more after the start of drug perfusion (P < 0.01, paired Student’s t-test). The mean latency from the start of current injection to the peak of the first spike was 278 ± 42 ms before epinephrine and 189 ± 21 ms after the start of drug perfusion (n = 11, P < 0.05). Of the 11 neurons tested, 9 (81%) showed a significant increase in spike number, and of those, 5 (45% of neurons tested) also showed a decrease (of ≥50 ms) in the spike latency. The mean resting membrane potential of these neurons was not changed by epinephrine (−60 ± 2 mV before epinephrine, −60 ± 2 mV after, n = 11, P > 0.05).

Propranolol blocked the sensitization of small-diameter...
DRG neurons by epinephrine. When 10 μM propranolol was perfused 30 s before and with 1 μM epinephrine, there was no increase in the number of spikes (1.2 ± 0.1 before drugs vs. 1.3 ± 0.2 after, n = 7, P > 0.05) nor any decrease in the latency to the first spike (235 ± 9 vs. 233 ± 10 ms, P > 0.05).

Because hyperalgesic agents (e.g., PGE2) that sensitize nociceptors in vitro have been shown to increase TTX-R INa (England et al. 1996; Gold et al. 1996b), we performed whole cell voltage-clamp experiments to determine whether epinephrine acted similarly. Epinephrine (1 μM) caused a marked potentiation of TTX-R INa (Fig. 5). The peak current amplitude in response to a depolarizing test pulse was increased by 37 ± 5% (n = 24, P < 0.01). Comparison of the current-voltage plots before and after drug exposure indicate that activation of TTX-R INa was shifted by ~10 mV in the hyperpolarized direction (Fig. 5B). This dose of epinephrine caused an increase in TTX-R INa in 16 of 24 neurons (67%); isoproterenol caused a similar increase in TTX-R INa (Fig. 5C). There was no potentiation of TTX-R INa when epinephrine was applied in the presence of 2 μM propranolol; mean normalized current was 87 ± 5% of baseline in the third minute of exposure to epinephrine and propranolol (n = 6) (Fig. 6). The increase in current was not attenuated by 5 μM phentolamine (n = 5) (Fig. 6).

In the behavioral experiments, hyperalgesia caused by epinephrine was dependent on both the PKA and PKC second-messenger systems. Therefore we examined the relative roles these two second-messenger systems play in the effect of epinephrine on TTX-R INa. When Rp-cAMPS (100 μM), a competitive inhibitor of PKA types I and II, was included in the recording pipette, the increase in TTX-R INa by epinephrine was prevented (Fig. 7).

We also applied epinephrine in the presence of the PKC inhibitor, BIM. The mean normalized current increase in response to 1 μM epinephrine was significantly smaller when neurons were pretreated with BIM compared with control experiments with no BIM pretreatment (Fig. 8; P < 0.01, n = 11 for both conditions). Of the control cells, 8 of 11 responded to the epinephrine (defined as a >10% increase in current amplitude within 3 min) whereas only 5 of 11 did with BIM present. For those neurons that responded to epinephrine, there was a mean increase of 49% in the peak current for the controls compared with 32% with BIM present. Therefore, inhibition of PKC caused both a decrease in the number of neurons responding to epinephrine and a decrease in the mean magnitude of the response.

**DISCUSSION**

The rapid onset of epinephrine hyperalgesia and the observation that neither sympathectomy nor indomethacin pretreatment affected it, is consistent with a direct action of epinephrine on sensory nerve terminals in the skin (Taiwo and Levine 1989b, 1990, 1992). The efficacy of our sympathectomy or indomethacin has been established in previous studies by observing an immediate increase in paw temperature of 2–3°C after sympathectomy as well as the loss of BK-induced hyperalgesia in the normal rat (Khasar et al. 1998; Miao et al. 1996). We have suggested earlier that small-diameter cultured DRG neurons...
neurons are a good in vitro model for the study of nociceptors (Gold et al. 1996a). Furthermore we have shown that agents, such as PGE2, that produce hyperalgesia by a direct action on primary afferents enhance TTX-R $I_{Na}$ in cultured DRG neurons (Gold et al. 1996b). The observed potentiation of TTX-R $I_{Na}$ by epinephrine may be the mechanism by which it sensitizes nociceptors (England et al. 1996; Gold et al. 1996b). The sensitivity of epinephrine- or isoproterenol-induced hyperalgesia to propranolol but not phentolamine is consistent with a $\beta$-AR-mediated effect. These $\beta$-ARs are most likely coupled to G proteins because epinephrine-induced hyperalgesia was attenuated by DAMGO. DAMGO previously has been shown to attenuate PGE2-induced hyperalgesia by activating a pertussis toxin-sensitive inhibitory G protein (Khasar et al. 1995b) and to prevent the potentiation of TTX-R $I_{Na}$ by PGE2 (Gold and Levine 1996); a similar mechanism may explain inhibition of epinephrine-induced hyperalgesia. Downstream from receptor activation, the production of cAMP as well as activation of both PKA and PKC were necessary because their respective antagonists inhibited epinephrine-induced hyperalgesia.

The results of the in vitro experiments on DRG neurons are consistent with the behavioral data and definitively demonstrate a direct action of epinephrine on primary afferent neurons. Both the sensitization of small-diameter neurons and the potentiation of TTX-R $I_{Na}$ by epinephrine, like behavioral hyperalgesia, were blocked by propranolol. The potentiation of TTX-R $I_{Na}$ was mimicked by isoproterenol. Taken together, these results provide the first evidence for $\beta$-ARs on DRG

FIG. 6. Normalized mean response of TTX-R $I_{Na}$ to epinephrine alone and in the presence of $\alpha$- or $\beta$-AR blockers. Peak inward current amplitude throughout each experiment was normalized to the average of the current during the last 3 min before the application of epinephrine. Time course of changes in the mean normalized current ± SE is plotted with data binned into 1-min intervals. In this and the following 2 figures, all experiments for a given condition were included in the data analysis, including those in which the current was unaffected by epinephrine (~20–30% of controls). Propranolol (2 $\mu$M, $n = 6$) or phentolamine (5 $\mu$M, $n = 7$) was perfused into the recording chamber 1 min before coperfusion of 1 $\mu$M epinephrine and inhibitor as indicated by the bars. Current increase was unaffected by phentolamine ($P > 0.05$) but was blocked by propranolol ($P < 0.01$) when compared with epinephrine alone ($n = 24$).

FIG. 7. Inhibition of the cAMP-dependent protein kinase prevents the increase in TTX-R $I_{Na}$ by epinephrine. Recordings were made with either the PKA inhibitor Rp-cAMPS (100 $\mu$M, $n = 5$) or sucrose (100 $\mu$M, $n = 5$) added to the electrode solution. Whole cell configuration was maintained for ~10 min before epinephrine was applied to allow Rp-cAMPS to diffuse into the cell from the electrode. There was no increase in TTX-R $I_{Na}$ when the inhibitor was present (for minutes 3–10 after onset of epinephrine perfusion) ($P < 0.05$) compared with epinephrine alone.

FIG. 8. Inhibition of protein kinase C (PKC) reduces the potentiation of TTX-R $I_{Na}$ by epinephrine. Mean normalized current during experiments in which the PKC inhibitor bisindolylmaleimide (BIM) was perfused into the chamber for 3 min before and during epinephrine ($n = 11$). Compared with epinephrine alone ($n = 11$), BIM significantly reduced the increase in TTX-R $I_{Na}$ ($P < 0.05$).
neurons as well as sensory nerve terminals. Potentiation of TTX-R $I_{Na}$ by epinephrine was blocked by inhibition of PKA but also significantly attenuated by inhibition of PKC. The mechanical hyperalgesia, sensitization, and potentiation of TTX-R $I_{Na}$ all occurred with a similar time course of onset after introduction of epinephrine, the effects peaking within 5 min.

Ferreira (1980) found that the methylxanthines, caffeine, and theophylline (which, among other actions, are inhibitors of phosphodiesterase, the enzyme that breaks down cAMP), potentiated the hyperalgesic effect of epinephrine and isoproterenol and concluded that the propranolol-sensitive hyperalgesia was mediated by the cAMP second-messenger system. Our data, from experiments using inhibitors of adenylyl cyclase, PKA and PKC, suggest that the $\beta$-AR-mediated hyperalgesia is mediated by the PKC as well as the PKA second-messenger systems because PKC as well as PKA inhibitors significantly attenuated epinephrine hyperalgesia. PKC has been shown to play a role in nociceptor sensitization, both in vivo and in vitro (Cesare and McNaughton 1996; Leng et al. 1996). Inhibition of isoproterenol-induced hyperalgesia by BIM and WPI/TIDE suggests that $\beta$-AR activation can be mediated by both the PKA and the PKC second-messenger systems. Thus $\beta$-AR activation alone is enough to account for the hyperalgesic action of epinephrine. The in vitro results agree with the behavioral data in that TTX-R $I_{Na}$ potentiation by epinephrine was blocked by Rp-cAMPS and significantly attenuated by BIM.

Epinephrine does not play a significant role as a neurotransmitter in the periphery or as a local inflammatory mediator under normal circumstances. However, its release from the adrenal medulla (its main source in the periphery) is increased by acute stress (Taylor et al. 1989; Wortsman et al. 1984) as is the activity in sympathetic postganglionic neurons (Mazzeo et al. 1997). Under conditions of acute stress, circulating levels of epinephrine have been shown to increase dramatically in humans as well as in rats (Cryer 1980; DeTurck and Vogel 1980; Taylor et al. 1989; Wortsman et al. 1984) and stress has been shown to induce hyperalgesia (Kawanishi et al. 1997; Okano et al. 1997; Vidal and Jacob 1986). Although it remains to be determined whether the epinephrine levels attained under these circumstances actually contribute to primary afferent nociceptor sensitization and hyperalgesia, increases in circulating levels of epinephrine produced by stress have well-documented physiological effects (Cryer 1980; Wortsman et al. 1984). Epinephrine is the endogenous $\beta$-AR agonist, and $\beta$-AR antagonists can attenuate inflammation in humans with rheumatoid arthritis (Kaplan et al. 1980) and in adjuvant-induced arthritis in the rat (Coderre et al. 1990; Levine et al. 1988). Furthermore, it has been suggested that epinephrine can produce cardiac pain (angina) independent of its vasoconstrictor effects in patients with syndrome X who have normal coronary arteries (Eriksson et al. 1995). Certainly, effects of epinephrine and the role of $\beta$-ARs in the mediation of pain and hyperalgesia deserve further study, both in the setting of nerve injury as well as inflammation, in which the sympathoadrenal axis may be activated.

In conclusion, our data suggest that epinephrine produces mechanical hyperalgesia in the rat and sensitizes cultured DRG neurons. These effects of epinephrine appear to be mediated by at least two second-messenger systems, cAMP/PKA and PKC.

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