Neural and Endocrine Mechanisms Mediating Noxious Stimulus-Induced Inhibition of Bradykinin Plasma Extravasation in the Rat

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ABSTRACT

We studied the mechanisms by which activation of primary afferent nociceptors inhibits bradykinin-induced plasma extravasation in the rat. First, capsaicin, administered into the plantar surface of the hindpaw, dose-dependently inhibited bradykinin-induced plasma extravasation in the knee joint, a site distant from the noxious stimulus. The inhibitory effect of capsaicin was markedly attenuated after T12/L1 spinal transection combined with lumbar preganglionic sympathetic denervation. Second, interruption of the spinal sympathetic and sympathoadrenal outflow. Second, interruption of the ascending spinal tracts to rostral sites and to spinal combined with lumbar preganglionic sympathectomy, which interrupts ascending spinal tracts to rostral sites and to spinal sympathetic and sympathoadrenal outflow. Second, interruption of the sympathoadrenal pathway produced the largest attenuation. Lesioning of the hypothalamic-pituitary-adrenal axis did not affect the inhibitory action of capsaicin. Third, intra-articular perfusion with phentolamine (10^{-5} M, an \alpha-adrenergic receptor antagonist), propranolol (10^{-5} M, a \beta-adrenergic receptor antagonist), and naloxone (10^{-5} M, an opioidergic receptor antagonist) each attenuated the inhibitory action of capsaicin. Propranolol and naloxone produced the largest attenuation. Blocking glucocorticoid receptors (RU-38,486, 30 mg/kg s.c.) did not affect the inhibitory action of intraplantar capsaicin. Fourth, the magnitude of the attenuation of capsaicin-induced inhibition of bradykinin-induced plasma extravasation after a combined treatment of surgical lumbar sympathetic decentralization with intra-articular phentolamine or surgical adrenal de-nervation with intra-articular propranolol or naloxone was similar to each of the surgical or pharmacological treatments of the same axis alone. These results support the suggestion that two neural/endocrine circuits, sympathoadrenal and sympathetic, account for most, if not all, of nociceptor activity-induced inhibition of bradykinin-induced plasma extravasation produced by capsaicin.

Although inflammation involves a cascade of events that function to ensure a rapid and effective response to injury, at the same time an appropriate modulation of the inflammatory response is needed to optimize the host response. Injury has a dual effect: one to cause local inflammation and the other to activate feedback systems to suppress inflammation. It is known that some substances (e.g., interleukin-1 and tumor necrosis factor) released during inflammation can stimulate the hypothalamic-pituitary-adrenal (HPA), sympathoadrenal, and sympathetic systems and thus suppress an inflammatory response (Bernton et al., 1987; Ichijo et al., 1994; Wociejewowsky et al., 1999). In addition to these hormonal pathways, evidence is accumulating that the activated nociceptive afferents transmit signals to the central nervous system to suppress inflammation (Sternberg et al., 1990; Wang et al., 1994; Sato, 1995; Zhang and Johns, 1997). Other stressors also induce activation of neural/endocrine systems to suppress inflammation (e.g., Lau, 1992; Lachuer et al., 1994; Malendowicz et al., 1994; Murakami et al., 1997). Therefore, we hypothesized that noxious stimuli generated at the site of injury initiates a feedback mechanism to down-regulate inflammation.

We recently characterized the contribution of several candidate neural/endocrine systems to feedback inhibition of inflammation. In our studies, a noxious stimulus was applied to one site (i.e., the hindpaw) to activate nociceptors. Plasma extravasation, a critical component of the inflammatory response, at a distal structure (i.e., the knee joint) was used as a model of inflammation to evaluate the control of inflammation by a noxious stimulus. We found that the anti-inflammatory effect produced by electrical stimulation of the paw is mediated by the HPA axis (Green et al., 1995). Because electrical stimulation activates non-nociceptive afferents as

ABBREVIATIONS: HPA, hypothalamo-pituitary-adrenal; BK, bradykinin; PE, synovial plasma extravasation.
well (Koizumi and Brooks, 1972; Sato and Schmidt, 1973), in the present study we used the selective C-fiber activator capsaicin to study the mechanisms mediating nociception-induced inhibition of inflammation.

In this study, we examined the contribution of these three neural/endocrine pathways to inhibition of bradykinin-induced synovial plasma extravasation (BK-induced PE) produced by noxious stimuli induced by injection of capsaicin into the hindpaw. Surgical and pharmacological approaches, shown schematically in Fig. 1, were used to interrupt neural/endocrine axes. In contrast to the effects of electrical stimulation (Green et al., 1995) or intrathecal nicotine (Miao et al., 1994, 1997c), the inhibitory pathway activated by intraplantar capsaicin is mediated, predominantly by the sympathoadrenal and sympathetic systems. The HPA axis does not significantly contribute to the inhibitory action of intraplantar capsaicin.

**Experimental Procedures**

The experiments were performed on male Sprague-Dawley rats (300–400 g). Rats were anesthetized by i.p. injection of sodium pentobarbital (65 mg/kg; Abbott Lab, Chicago, IL) before surgical procedures or knee joint perfusion experiments. Animal care and use conformed to the guidelines of the National Institutes of Health for the care and use of experimental animals. Experimental protocols were approved by the University of California at San Francisco Committee on Animal Research.

**Perfusion of Knee Joint**

Knee joint perfusion was performed as described previously (Coderre et al., 1989; Miao et al., 1996a). In brief, after incision of the skin and connective tissue overlying the anterior aspect of the knee and the saphenous vein, Evans blue dye (50 mg/kg i.v.) was administered. Ten minutes later, a 30-gauge needle was inserted into the cavity of the knee joint for the infusion of fluid (250 μl/min). After infusion of an initial volume of 100 to 200 μl of vehicle, a second needle (25 gauge), serving as an outflow cannula, was inserted into the knee joint, approximately 3 mm from the inflow needle. Fluid was withdrawn from the joint through the outflow cannula with the use of a second syringe pump. The fluid was infused and withdrawn at a constant rate of 250 μl/min. Perfusion samples were collected every 5 min for up to 145 min. Samples were analyzed for the amount of Evans blue dye through spectrophotometric measurement of absorbance at 620 nm. The absorbance at this wavelength is linearly related to the dye concentration (Carr and Wilhelm, 1964).

After a baseline perfusion period of 15 min with vehicle (normal saline, first three readings in Fig. 2), plasma extravasation into the knee joint was stimulated by the addition of BK (160 ng/ml, i.e., 0.15 μM) to the perfusion fluid. Of note, the concentration of BK in various inflamed tissues is in the range of 50 nM to 0.1 μM (Hargreaves et al., 1995; Swift et al., 1993). Both knee joints in the same rat were perfused simultaneously. For some experiments in which receptor antagonists (except glucocorticoid receptor antagonist, which is not soluble in perfusion fluid) were used, one knee was perfused with receptor antagonist and BK, and the contralateral knee was perfused with vehicle and BK.

**Noxious Stimulation of Primary Afferents by Intraplantar Capsaicin**

To initiate nociceptor-induced inhibition of plasma extravasation, spinal afferents were excited from a site remote from the knee (i.e., hindpaw) with intraplantar injection of capsaicin. For some experiments in which function of supraspinal sites in noxious stimulus-induced inhibition of PE was tested, afferents from the forepaw were activated by intrapalmar capsaicin. Capsaicin was injected in the paw at progressively higher doses (3–100 μg, at half-log dose increments in volume of 10 μl each) at intervals of 20 min.

**List of Experimental Groups**

**Sham Surgery Control.** To compare the effect of surgical ablation, sham surgery was performed as a control. In this group of animals, the sham surgery was performed by cutting the cutaneous and muscular layers of the abdomen and then closing the wound, as in the experimental groups. The intra-abdominal site where the lesion was made in the experimental groups was not manipulated.

**Transection of Sciatic and Saphenous Nerves.** To examine whether the effect of intraplantar capsaicin is mediated exclusively through the nerves innervating the hindpaw, we transected the ipsilateral sciatic and saphenous nerves immediately before knee joint perfusion experiments. After separation of the biceps femoris and semitendinosus muscles at the posterior aspect of the thigh, the sciatic nerve was located by blunt dissection. It was cut at a level close to that of the sacral plexus (before it gives rise to the tibial and common peroneal nerves). The saphenous nerve was isolated from...
the adjacent vascular bundle on the medial aspect of the thigh and cut at a level just superior to its trifurcation. Eight knees from four rats were used in this experiment. Transection of sciatic and saphenous nerves did not affect the baseline level of BK-induced PE (Table 1).

Spinal Transection (T12/L1 and T12/L1). To examine the contribution of spinal pathways to the noxious stimulus-induced inhibition of PE, the spinal cord was exposed by laminectomy and then transected immediately before knee joint perfusion experiments; Gelfoam was inserted into the lesion site to separate the cut spinal sections.

The adrenal medulla receives preganglionic sympathetic innervation from the T3–10 spinal level (Kesse et al., 1988; Parker et al., 1990). Therefore, to separately examine the contribution of spinal mechanisms (i.e., to the adrenal medulla and lumbar sympathetic) and those of rostral sites, in different groups of rats spinal transection was performed at either of two levels: T12/L1, where spinal pathways to both the adrenal medulla and supraspinal sites were interrupted, and T1/L2, where spinal pathways to the rostral sites were transected but the connections to the adrenal medulla were spared. Spinal transection at T1/L2 level did not affect the baseline level of BK-induced PE (Table 1) as reported previously (Miao et al., 1994). Spinal transection at T12/L1 level decreased the baseline level of BK-induced PE (Table 1). Sixteen knees from eight rats were used in each of these experiments.

T1/L2. Spinal Transection plus L1–3 Lumbar Sympathetic Decentralization. T1/L2 spinal transection alone can eliminate input from hindlimb nociceptors to supraspinal and spinal sympathoadrenal circuits. However, the level of this surgery is above spinal circuits projecting to the intermediolateral column at L1–3 (Celler and Schramm, 1981), preserving lumbar sympathetic outflow. Therefore, a combined surgery of T1/L2 spinal transection and bilateral L1–3 lumbar sympathetic decentralization was conducted. This surgical procedure, like T12/L1 spinal transection, decreased the baseline level of BK-induced PE (Table 1). Eight knees from four rats were used in this experiment.

Hypophysectomy. Hypophysectomized rats and controls were purchased commercially from Charles River (Hollister, CA). These animals were used in plasma extravasation experiments 2 weeks after surgery. BK-induced PE in these hypophysectomized rats was not significantly different from that in normal Sprague-Dawley rats from Charles River. Hypophysectomy did not affect the baseline level of BK-induced PE (Table 1) as reported previously (Miao et al., 1993). Bilateral adrenal nerve lesion did not affect the baseline level of BK-induced PE (Table 1). Eight knees from four rats were used in this experiment.

Subcutaneous Administration of RU-38,486. To examine the contribution of glucocorticoids to nociceptive activity-induced inhibition of BK-induced PE, we pretreated rats, 3 h before the commencement of the knee joint perfusion, with RU-38,486 (30 mg/kg s.c.), a glucocorticoid and progesterone receptor antagonist (Peeters et al., 1992; Weinstein et al., 1992). A similar protocol using RU-38,486 at this dose has been found to be effective in antagonizing actions mediated by glucocorticoid receptors in other systems (Peeters et al., 1992). RU-38,486 did not affect the baseline level of BK-induced PE (Table 1) as reported previously (Miao et al., 1997b). Eight knees from four rats were used in this experiment.

Intra-articular Administration of Phentolamine. To study the contribution of α-adrenoceptors to the nociceptive activity-generated inhibition of BK-induced PE, we coperfused phentolamine, an α-adrenoceptor antagonist, into the knee joint at a concentration of 10⁻⁶ M. The concentration we used in this experiment was adopted from reports that showed that at this concentration, phentolamine is effective in antagonism of the α-adrenoceptor-activating action of norepinephrine (Bush et al., 1990; Krupin et al., 1991). Phentolamine, at the concentration used in this experiment, did not affect the baseline level of BK-induced PE (Table 1). Eight knees were obtained from eight rats each for the ipsilateral and contralateral groups.

Intra-articular Administration of Propranolol. To study the contribution of β-adrenoceptors to the nociceptive activity-generated depression of BK-induced PE, the β-adrenoceptor antagonist propranolol was coperfused into the knee joint at a concentration of 10⁻⁵ M, which has been shown to be an effective concentration in antagonizing actions of isoproterenol (Tanaka et al., 1995). Propranolol, at the concentration used in this experiment, did not affect the baseline level of BK-induced PE (Table 1). Eight knees were obtained from eight rats each for the ipsilateral and contralateral groups.

Intra-articular Administration of Naloxone. To study the contribution of endogenous opioids to nociceptive activity-induced inhibition of BK-induced PE, naloxone, an opioidergic receptor antagonist, was coperfused with BK into the knee joint. Naloxone was administered at a concentration of 10⁻⁵ M, a concentration previously reported to be effective in blocking opioidergic receptors (Taguchi et al., 1990). Naloxone did not affect the basal level of BK-induced

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**TABLE 1**

<table>
<thead>
<tr>
<th>Group</th>
<th>Maximum BK-Induced PE</th>
<th>n</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.141 ± 0.010</td>
<td>8</td>
</tr>
<tr>
<td>Deafferentation</td>
<td>0.176 ± 0.018</td>
<td>8</td>
</tr>
<tr>
<td>T1 spinalization</td>
<td>0.192 ± 0.008</td>
<td>8</td>
</tr>
<tr>
<td>L1 spinalization</td>
<td>0.116 ± 0.097</td>
<td>8</td>
</tr>
<tr>
<td>L1 spinalization + sympathetic decentralization</td>
<td>0.104 ± 0.009</td>
<td>8</td>
</tr>
<tr>
<td>Adrenal denervation</td>
<td>0.157 ± 0.020</td>
<td>8</td>
</tr>
<tr>
<td>Sympathetic decentralization</td>
<td>0.137 ± 0.009</td>
<td>8</td>
</tr>
<tr>
<td>Hypophysectomy</td>
<td>0.136 ± 0.012</td>
<td>8</td>
</tr>
<tr>
<td>RU-38, 486</td>
<td>0.142 ± 0.014</td>
<td>8</td>
</tr>
<tr>
<td>Hypophysectomy + RU-38, 486</td>
<td>0.151 ± 0.022</td>
<td>8</td>
</tr>
<tr>
<td>Phentolamine</td>
<td>0.150 ± 0.014</td>
<td>8</td>
</tr>
<tr>
<td>Phentolamine + phentolamine</td>
<td>0.152 ± 0.012</td>
<td>8</td>
</tr>
<tr>
<td>Propranolol</td>
<td>0.177 ± 0.020</td>
<td>8</td>
</tr>
<tr>
<td>Adrenal denervation + propranolol</td>
<td>0.123 ± 0.007</td>
<td>8</td>
</tr>
<tr>
<td>Naloxone</td>
<td>0.166 ± 0.018</td>
<td>8</td>
</tr>
<tr>
<td>Naloxone + naloxone</td>
<td>0.137 ± 0.009</td>
<td>8</td>
</tr>
</tbody>
</table>

n, number of knee joint preparations.

* No significant difference from the data obtained in the control (P > .05).

* Significantly different from the data obtained in the control (P < .05).

BK-induced PE is measured as absorbance at 620 nm, which is linearly related to the concentration of circulating Evans blue dye.
PE (Table 1) as reported previously (Miao et al., 1997a). Naloxone, at the concentration used in this experiment, did not affect the baseline level of BK-induced PE (Table 1). Eight knees were obtained from eight rats each for the ipsilateral and contralateral groups.

**Lumbar Sympathetic Decentralization plus Intra-articular Phentolamine.** To determine whether the lumbar sympathetic outflow is the source of a-adrenergic agonist to mediate nociceptive activity-induced inhibition of BK-induced PE, we perfused phentolamine (10^{-5} M) into the knee of rats whose preganglionic sympathetic fibers in the lumbar sympathetic chains had been transected. This combination of treatment did not affect the baseline level of BK-induced PE (Table 1). Eight knees from four rats were used in this experiment.

**Adrenal Denervation plus Intra-articular Propranolol or Naloxone.** To determine whether the endogenous \( \beta \)-adrenergic agonist that mediates inhibition of BK-induced PE by nociceptive activity is from the adrenal medulla, we perfused the knee with propranolol (10^{-5} M) in bilateral adrenal denervated rats. This combined treatment did not affect the baseline level of BK-induced PE (Table 1). Eight knees from four rats were used in this experiment.

To examine whether the majority of the endogenous opioids mediating the anti-inflammatory action of nociceptive activity is from the adrenal medulla, we intra-articularly perfused naloxone (10^{-5} M) into the knee of bilateral adrenal denervated rats. This combined treatment did not affect the baseline level of BK-induced PE (Table 1). Eight knees from four rats were used in this experiment.

**Experimental Procedures and Statistics**

Data are presented as mean ± S.E.; two-way (group × time for time-effect curves or group × dose for dose-response curves) repeated measures ANOVA was used to determine significant differences between pairs of curves. Differences were considered statistically significant at a value of \( P < .05 \). Whenever a receptor antagonist was perfused into one knee, the contralateral knee was always used to compare the response with that in the knee perfused with antagonist. The contralateral knee was also compared with the normal control to ensure they were similar. If surgical intervention was used, knees from sham controls were used for the comparison.

**Dose-Response Relationships**

Dose-response relationships for intraplantar capsaicin inhibition of BK-induced PE were obtained by a cumulative dosing method (Miao and Lee, 1989; Miao et al., 1997b). Data for 300 \( \mu \text{g} \) of capsaicin, which induced maximal inhibition (i.e., \( E_{\text{max}} \)), was not included because of apparent systemic effects (see Results). Therefore, \( ED_{50} \) values of experimental curve were not obtainable.

**Materials**

BK acetate, naloxone hydrochloride, propranolol hydrochloride, capsaicin, and Tween 80 were obtained from Sigma Chemical Co. (St. Louis, MO). Phentolamine hydrochloride was purchased from Ciba Pharmaceutical (Summit, NJ). RU-38,486 was a generous gift from Organon (Oss, The Netherlands). RU-38,486 was dissolved in peanut oil. All other chemicals were dissolved in normal saline.

**Results**

**Intra-articular Perfusion of BK-Induced PE.** Similar to results reported previously (Coderre et al., 1989; Miao et al., 1993), the continuous infusion of BK (160 ng/ml, 0.15 \( \mu \text{M} \)) through the knee joint increased synovial plasma extravasation (Fig. 2). This increase was time dependent, reaching a plateau after 30 min of perfusion and then decreasing slightly over time. At the end of the experiment (145 min), the decay from the peak response (crosses in this and subsequent figures) was 10.6 ± 3.2% in sham surgery animals.

**Intraplantar Capsaicin Dose-Dependently Inhibited BK-Induced PE.** To initiate nociceptor-induced inhibition of plasma extravasation in the knee joint, spinal afferents were excited at a site (i.e., hindpaw) remote from the knee, by intraplantar injection of capsaicin. The intradermal administration of capsaicin, in cumulatively higher doses, into the hindpaw produced a dose-dependent decrease in BK-induced PE (Figs. 2 and 3, open circles; \( P < .01 \), one-way ANOVA). The time-effect curve of the intraplantar capsaicin group was significantly different from that of the control group (crosses; \( F_{20,300} = 21.0 \), two-way ANOVA, \( P < .01 \)). Intradermal capsaicin also decreased PE induced by platelet-activating factor (data not shown), a non-neurogenic inflammatory mediator (Green et al., 1993).

**Transsection of Sciatic and Saphenous Nerves Abolished Effect of Capsaicin.** To determine whether the effect of intraplantar capsaicin is mediated exclusively through activation of the nerves innervating the lower limbs, we examined the effect of unilateral transection of sciatic and saphenous nerves on intraplantar capsaicin-induced inhibition of BK-induced PE. Acute transection of these nerves (Fig. 2, filled circles) almost completely abolished the inhibitory effect of capsaicin (except at 300 \( \mu \text{g} \), the highest dose; cross versus filled circles, \( F_{20,300} = 5.1 \), two-way ANOVA, \( P < .05 \)).

**\( T_2/\text{T}_3 \) Spinal Transection Attenuated Effect of Capsaicin.** To distinguish mechanisms for inhibition of BK-induced PE mediated by supraspinal circuits from those mediated by spinal circuits, we evaluated the effects of high-level spinal transection (i.e., at the \( T_2/\text{T}_3 \) level) on capsaicin-induced inhibition of BK-induced PE (Fig. 3, filled circles). Acute spinal transection at \( T_2/\text{T}_3 \) produced no significant attenuation of the capsaicin inhibition of BK-induced PE (filled circles versus open circles, \( F_{3,45} = 0.04 \), \( P > .05 \), two-way ANOVA).

**\( T_{12/L_1} \) Spinal Transection Attenuated Effect of Capsaicin.** To examine the contribution of supraspinal circuits to the inhibition of BK-induced PE, we next evaluated the effects of \( T_{12/L_1} \) spinal transection on the inhibitory effect of capsaicin (Fig. 3, half-filled circles). Acute spinal transection at \( T_{12/L_1} \) produced an attenuating effect of the capsaicin inhibition of BK-induced PE compared with that of the control group (half-filled circles versus open circles, \( F_{3,45} = 9.05 \), two-way ANOVA, \( P < .01 \)). The rightward shift in the curve produced by \( T_{12/L_1} \) spinal transection was greater than that after \( T_2/\text{T}_3 \) spinal transection (filled circles versus half-filled circles, \( F_{3,42} = 6.62 \), two-way ANOVA, \( P < .05 \)). At the end of the experiment, nociceptive pathways were activated above the \( T_2/\text{T}_3 \) level by capsaicin administration into the forepaw to test whether the spinal inhibitory mechanisms are still intact; forepaw injection of capsaicin markedly decreased BK-induced PE (unconnected half-filled circle).

**\( T_{12/L_1} \) Spinal Transection plus Lumbar Sympathetic Decentralization (\( L_1-3 \)) Eliminated Effect of Capsaicin.** The dose-response curve in the spinal transection plus sympathetic decentralization group (Fig. 3, crossed squares) was significantly different from that in the sham control group (crossed squares versus open circles, \( F_{2,45} = 7.14 \), two-way ANOVA, \( P < .01 \)). This curve, however, was not significantly different from that of \( T_{12/L_1} \) spinal transection group (half-
filled circles versus crossed squares; $F_{3,42} = 0.55$, $P > .05$, two-way ANOVA) but was significantly different from that of the T$_1$/T$_2$ spinal transection group (crossed squares versus filled circles, $F_{3,42} = 8.72$, $P < .01$). Capsaicin was injected into the plantar surface of the forepaw at the end of the experiment and produced a decrease in BK-induced PE (crossed square; $P < .01$).

**Interruption of HPA Axis Did Not Attenuate Effect of Capsaicin.** We next tested the hypothesis that a supraspinal component of capsaicin-induced inhibition of BK-induced PE is mediated by the HPA axis. Surgical hypophysectomy (Fig. 4, filled diamonds) did not significantly attenuate capsaicin-induced depression of BK-induced PE compared with the sham control group (filled diamonds versus open circles, $F_{3,45} = 0.70$, $P > .05$, two-way ANOVA).

**Interruption of Lumbar Sympathetic System Attenuated Effect of Capsaicin.** We further tested the hypothesis that a spinal component of capsaicin-induced inhibition of BK-induced PE is mediated by the sympathoadrenal axis. Sympathetic preganglionic innervation to the adrenal gland was interrupted by suprarenal ganglionectomy. Adrenal denervation markedly attenuated the inhibition of BK-induced PE generated by intraplantar capsaicin (Fig. 4, half-filled circles). The intraplantar capsaicin dose-response curve in the adrenal denervation group was significantly different from the sham surgery control (half-filled circles versus open circles, $F_{3,45} = 5.45$, $P < .05$, two-way ANOVA).

**Glucocorticoid Receptor Antagonist RU-38,486 Did Not Significantly Attenuate Effect of Capsaicin.** We evaluated the role of the final common mediator of the HPA axis in the rat, glucocorticoid, in capsaicin-induced inhibition of BK-induced PE. RU-38,486, a glucocorticoid and progesterone receptor antagonist, did not significantly affect capsaicin inhibition of BK-induced PE (Fig. 5, filled circles; filled circles versus open circles, $F_{3,45} = 2.84$, $P < .05$, two-way ANOVA).

**α-Adrenoceptor Antagonist Phenolamine Attenuated Effect of Capsaicin.** We examined the effect of locally administered phenolamine, an antagonist for α-adrenoceptors, on the inhibitory action of capsaicin. Intra-articularly perfused phenolamine ($10^{-5}$ M), into the knee joint, attenuated capsaicin-induced inhibition of BK-induced PE (Fig. 6, filled triangles). As a control, the contralateral knee was
perfused with BK plus vehicle (open triangles). The dose-response curve for intraplantar capsaicin in phentolamine-treated knees was shifted to the right (filled triangles versus open triangles, $F^{3,42}_{3,42} = 12.50, P < .01$, two-way ANOVA). The inhibitory effect of intraplantar capsaicin in the contralateral control knees and that of the sham surgery controls were similar in their dose-response curves (open triangles versus open circles, $F^{3,45}_{3,45} = 0.58, P > .05$, two-way ANOVA).

**Phentolamine Produced No Additional Attenuation of Effect of Capsaicin in Surgically Sympathetic-Decentralized Rats.** To determine the contribution of $\alpha$-adrenoceptors to postganglionic lumbar sympathetic neuron-mediated inhibition of BK-induced PE, we combined surgical lumbar sympathetic decentralization with intra-articular phentolamine (Fig. 6, filled circles). The capsaicin dose-response curve in this group (i.e., lumbar sympathetic decentralization plus phentolamine) overlapped with that for lumbar sympathetic decentralization alone (half-filled circles versus filled circles or versus filled triangles, $F^{3,42}_{3,42} = 0.75$ and 3.89, respectively, both $P > .05$, two-way ANOVA).

**$\beta$-Adrenoceptor Antagonist Propranolol Attenuated Effect of Capsaicin.** To determine the contribution of $\alpha$- and $\beta$-adrenoceptors to capsaicin-induced inhibition of BK-induced PE, we first examined the effect of locally administered propranolol, an antagonist for $\beta$-adrenoceptors, on the inhibitory action of capsaicin.

Compared with contralateral control knees, which were perfused with BK plus vehicle (open triangles), the dose-response curve for intraplantar capsaicin in phentolamine-treated knees was shifted to the right (filled triangles versus open triangles, $F^{3,42}_{3,42} = 12.50, P < .01$, two-way ANOVA). The inhibitory effect of intraplantar capsaicin in the contralateral control knees and that of the sham surgery controls were similar in their dose-response curves (open triangles versus open circles, $F^{3,45}_{3,45} = 0.58, P > .05$, two-way ANOVA).

**Propranolol Produced No Additional Attenuation of Effect of Capsaicin Inhibition in Surgically Adrenal Denervated Rats.** To determine the contribution of $\beta$-adre-
noceptor agonists to the sympathoadrenal-mediated inhibition of BK-induced PE, we combined surgical adrenal denervation with intra-articularly perfused propranolol (Fig. 7, filled circles). The dose-response curve for intraplantar capsaicin in the combination treatment group (i.e., adrenal denervation plus propranolol) was similar to that of adrenal denervation alone (filled circles versus half-filled circles, $F_{3,42} = 0.44, P > .05$, two-way ANOVA) but different from that for propranolol alone (filled circles versus open triangles, $F_{3,42} = 14.67, P < .05$, two-way ANOVA).

**Opioidergic Receptor Antagonist Naloxone Attenuated Effect of Capsaicin.** We evaluated the role of endogenous opioids, known to be released by the sympathoadrenal system, as well as other tissues, to capsaicin-induced inhibition in BK-induced PE; we used naloxone perfused locally into the knee joint to demonstrate opioidergic involvement in capsaicin inhibition of BK-induced PE. Intra-articular naloxone ($10^{-5}$ M) attenuated the inhibitory effects of capsaicin (Fig. 8, filled triangles) compared with the contralateral knees, which were perfused with BK plus vehicle (open triangles; filled triangles versus open triangles, $F_{3,42} = 8.95, P < .01$, two-way ANOVA). The capsaicin dose-response curve in naloxone-treated knees was shifted to the right. The dose-response curve for the effect of intraplantar capsaicin on BK-induced PE in the contralateral knee was similar to that of the sham surgery controls (open triangles versus open circles, $F_{3,45} = 0.18, P > .05$, two-way ANOVA).

**Naloxone Produced No Additional Attenuation of Effect of Capsaicin in Surgically Adrenal Denervated Rats.** To determine the contribution of opioidergic receptor agonists to the sympathoadrenal-mediated inhibition of BK-induced PE, we combined surgical adrenal denervation with intra-articular perfusion of naloxone (Fig. 8, filled circles). The capsaicin dose-response curve in the adrenal denervation-plus-naloxone group was similar to that of adrenal denervation alone or that of naloxone alone (filled circles versus open triangles or versus half-filled circles, $F_{3,42} = 0.72$ and $2.17$, respectively, both $P > .05$, two-way ANOVA).

**Discussion**

Infection or trauma induces an inflammatory response via local release of proinflammatory substances (e.g., substance
P, calcitonin gene-related peptide, and so on) to improve wound healing and antimicrobial defense. However, lack of feedback control can result in inflammatory diseases. To prevent such a potentially harmful outcome, the inflammatory response is restrained by modulatory systems. In this study, we examined physiological systems that modulate the inflammatory response in the knee joint by using intraplantar capsaicin, a C-fiber excitotoxin. We demonstrated that the inhibition of BK-induced PE by intraplantar capsaicin was attenuated compared with the contralateral control group, which received no propranolol (Δ, n = 8) and significantly shifted the dose-response curve to the right (▲ versus Δ). Effect of combination of surgical adrenal denervation with intra-articular propranolol (10⁻⁵ M) on the depression of BK-induced PE generated by intraplantar capsaicin. Combined surgical intervention of the adrenal innervation and pharmacological antagonism of the adrenergic receptors (●, n = 8) did not generate an attenuation greater than that of the adrenal denervation alone (● versus Δ) but greater than that of propranolol alone (● versus half-circles). That at least two of the three candidate neural/endocrine systems examined (i.e., the sympathetic and sympathoadrenal systems) contribute to the anti-inflammatory action generated by the capsaicin-initiated mechanism. These observations were unexpected because our previous studies showed the HPA axis, not the sympathetic or sympathoadrenal systems, mediate the depression of BK-induced PE by C-fiber strength electrical stimulation of the hindpaw (Green et al., 1995) and the inhibitory effects of intrathecal nicotine (Miao et al., 1994, 1996b), a stimulus believed to activate nociceptive ascending pathway in the spinal cord (Rogers and Iwamoto, 1993). Of note, although capsaicin selectively stimulates the C-fiber population of the nociceptive primary afferents, high-intensity electrical stimulation activates A-fiber afferents as well as nociceptors, which have been shown to be sympatheinhibitory (Koizumi and Brooks, 1972; Sato and Schmidt, 1973) and therefore may mask part of the
effect of C-fiber activation. Nicotine stimulates spinal antinoceceptive pathways (Rogers and Iwamoto, 1993), as well as nociceptive pathways (Morita and Katayama, 1989; Franco-Cereceda et al., 1992; Khan et al., 1994), and therefore may also decrease sympathetic outflow. We have also found that peripherally administered nicotine, which activates C-fiber afferents (Steen and Reeh, 1993), decreases BK-induced PE, an effect dependent on the HPA (Miao et al., 1997a), as well as the sympathoadrenal (Miao et al., 1994, 1997a), axis. Of note, electrical stimulation of somatic nerves or injection of inflammatory mediators activates the HPA axis (Sternberg et al., 1990), the sympathetic nervous system, and the sympathoadrenal system (Wang et al., 1994; Sato, 1995; Zhang and Johns, 1997). These three neural/endocrine systems are also known to be activated by various other stressors (Lau, 1992; Lachuer et al., 1994; Malendowicz et al., 1994; Murakami et al., 1997). Which features of each stimulus lead to the activation of specific stress axes is unknown.

It should be pointed out that our study was conducted when the neural/endocrine systems were activated by a noxious stimulus. Therefore, the attenuation of the inflammatory response produced by capsaicin, after surgical/pharmacological intervention, does not imply an anti-inflammatory role of basal activity in these systems. In fact, evidence from our previous studies indicates no influence on BK-induced PE under basal conditions (Green et al., 1995; Miao et al., 1996b, 1997a). These findings suggest that these neural/endocrine systems mediate, not counteract, the inhibitory action of noxious stimuli.

In pharmacological intervention experiments, we introduced receptor antagonists into one knee joint and used the contralateral knee as a control, with the exception of the glucocorticoid receptor antagonist, which had to be administered systemically. Our results indicate that the site of action of the mediators (except for glucocorticoid, which remains to be determined) is locally in the knee joint. However, we do not know the cellular location of these receptors.

Although the local α-adrenoceptor-mediating neurovascular function and/or inhibition of BK-induced PE by noxious stimuli is likely to be postsynaptic (Elsner et al., 1986), as a constrictor of the precapillary sphinkters (Hirst et al., 1992), the action for β-adrenoceptor action is believed to occur via presynaptic inhibition (Encabo et al., 1996) of the release of neural mediators (e.g., both prostaglandin E₂ and ATP increase synovial PE) from sympathetic postganglionic neuron terminals (Coderre et al., 1989). Taken together, these observations suggest that both the sympathetic system, via release of norepinephrine, which can activate postsynaptic α-adrenoceptors to decrease blood flow through the synovial microcirculation, and the sympathoadrenal system, via epinephrine, which stimulates presynaptic β-adrenoceptors to reduce the release of neural mediators that promote PE, mediate a decrease in synovial PE.

The mechanism by which endogenous opioids regulate synovial PE has not been well established. Because the actions of opioidergic receptors tend to be inhibitory and opioidergic agonists decrease the release of neurotransmitters from SPG terminals and primary afferents (Khalil and Helme, 1990; Przewlocki et al., 1992), opioids may also act like epinephrine to decrease the release of neural mediators that promote PE. It was suggested that opioids can be released from immunocytes by corticotropin-releasing factor and thus mediate the inhibition of nociceptive afferents (for a review, see Mafer et al., 1997). However, because hypophysectomy and RU-38,486 as used in the present study interfere only with the release of adrenocorticotropic and activation of receptors for glucocorticoids, it is not known whether this corticotropin-releasing factor-opioid mechanism plays a role in our intraplantar capsaicin model.

Because endogenous opioids are not exclusively from an adrenal source, an extra-adrenal source of opioids released by high-dose capsaicin could contribute. Although the amount of opioids released from the central nervous system is unlikely to be sufficient to produce such effects via the circulating system, opioids from cells of the immune system, on stimulation, can produce physiological effects (Parsons et al., 1990; Przewlocki et al., 1992). Another possibility is an interaction between the adrenergic and opioidergic systems. Without restraint from a functional sympathoadrenal system, extra-adrenal opioids may contribute more to the inhibitory action of a noxious stimulus.

In conclusion, selective activation of C-fiber primary afferents by the excitotoxin capsaicin activates two mechanisms to inhibit BK-induced PE, a critical component of the inflammatory response, in the knee joint of the rat. To activate these mechanisms, nociceptive signals project to spinal efferent circuits (the sympathoadrenal and sympathetic systems) and to a supraspinal efferent circuit (the HPA axis). Among the hormones released by these systems, catecholamines, opioids, and glucocorticoids play important roles in the inhibition of inflammatory responses. Although it was not possible to examine the site of action for glucocorticoids, the knee joint was found to be the site of action for the catecholamines and opioids. We also found that the magnitude of the attenuation of the inhibitory effect of capsaicin was greater after surgical or pharmacological intervention on the sympathoadrenal system compared with that of intervention on the HPA axis and sympathetic nervous system.

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References


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