INTRODUCTION

Inflammation triggered by substances released from sensory nerve terminals is termed neurogenic inflammation. Components of neurogenic inflammation include arteriolar vasodilation (flare) and edema due to plasma extravasation from post-capillary venules (Brain et al. 1985; Majno et al. 1969; Szolcsanyi 1996). Antidromic activation of sensory axons in dorsal roots has long been known to produce vasodilation (Bayliss 1901). Acute inflammation after intradermal injection of capsaicin (CAP) is neurogenic (Szolcsanyi 1996) because it is associated with the activation of CAP-sensitive nociceptors and does not occur if the tissue is denervated or the nociceptors are desensitized by CAP pretreatment (Jancso et al. 1967, 1968; Lundblad et al. 1987).

We have demonstrated in anesthetized rats that much of the vasodilation and edema that result from CAP injection into the skin depends on spinally mediated activity (dorsal root reflexes, DRRs) conducted antidromically in primary afferent fibers (Lin et al. 1999, 2000a; reviewed by Willis 1999). Evidence for this includes the observations that most of the flare and edema after CAP injection is prevented by interruption of peripheral nerves, dorsal rhizotomies, intrathecal administration of glutamate receptor antagonists [6-cyano-7-nitroquinoxalene-2,3-dione (CNQX) and 2-amino-7-phosphonooctanoic acid (AP7)] that would block activation of GABAergic interneurons by afferent volleys, or intrathecal administration of bicuculline, an antagonist of GABA_A receptors, which are responsible for triggering DRRs (Lin et al. 1999, 2000a). Similar evidence has previously shown that DRRs account for a substantial part of the neurogenic inflammation seen in experimental arthritis (Rees et al. 1994, 1995; Sluka and Westlund 1993; Sluka et al. 1993).

The primary afferents that produce antidromic vasodilation are mainly C nociceptors, although Aδ nociceptors also contribute (Jänig and Lisney 1989; Lewin et al. 1992; Magerl et al. 1997). Our recent experiments have shown that the axons of Aδ and C afferents do conduct DRRs and that intradermal injection of CAP increases DRR activity in these axons (Lin et al. 2000b). DRRs in many nociceptive afferents would release inflammatory agents from injured or inflamed tissue. For instance, chemical sympathectomy (Bjerknes et al. 1991). The development of inflammatory responses by interaction with primary afferent terminals (Jañig et al. 1996; Michaelis 2000). The ability of sympathetic efferents to modulate neurogenic inflammation after intradermal injection of capsaicin (CAP) is mediated by dorsal root reflexes (DRRs), which cause the release of inflammatory agents from primary afferent terminals. Sympathetic efferents modulate neurogenic inflammation by interaction with primary afferent terminals. In this study, we examined if DRR-mediated flare after CAP injection is subject to sympathetic modulation. Changes in cutaneous blood flow on the plantar surface of the foot of sympathectomized rats was pretreated with an α_2-adrenoceptor agonist (phenylephrine) by intra-arterial injection, the spread of flare induced by CAP injection could be restored. However, if the spinal cord was pretreated with a GABA_A receptor antagonist, bicuculline, to prevent DRRs, phenylephrine no longer restored the CAP-evoked flare. An α_2-adrenoceptor agonist (UK14,304) did not affect the CAP-evoked flare in sympathectomized rats. In sympathetically intact rats, blockade of peripheral α_2-adrenoceptors with terazosin profoundly reduced the flare induced by CAP injection, whereas blockade of peripheral α_2-adrenoceptors by yohimbine did not obviously affect the flare. Therefore the pathogenesis of acute neurogenic inflammation in the intradermal CAP injection model depends in part on intact sympathetic efferents and α_2-adrenoceptors. Peripheral α_2-adrenoceptors thus modulate the ability of capsaicin sensitive afferents to evoke the release of inflammatory agents from primary afferents by DRRs.

Lin, Qing, Xiaoju Zou, Li Fang, and William D. Willis. Sympathetic modulation of acute cutaneous flare induced by intradermal injection of capsaicin in anesthetized rats. J Neurophysiol 89: 853–861, 2003; 10.1152/jn.00568.2002. Much of the acute cutaneous neurogenic inflammation after intradermal injection of capsaicin (CAP) in rats is mediated by dorsal root reflexes (DRRs), which cause the release of inflammatory agents from primary afferent terminals. Sympathetic efferents modulate neurogenic inflammation by interaction with primary afferent terminals. In this study, we examined if DRR-mediated flare after CAP injection is subject to sympathetic modulation. Changes in cutaneous blood flow on the plantar surface of the foot of sympathectomized rats was pretreated with an α_2-adrenoceptor agonist (phenylephrine) by intra-arterial injection, the spread of flare induced by CAP injection could be restored. However, if the spinal cord was pretreated with a GABA_A receptor antagonist, bicuculline, to prevent DRRs, phenylephrine no longer restored the CAP-evoked flare. An α_2-adrenoceptor agonist (UK14,304) did not affect the CAP-evoked flare in sympathectomized rats. In sympathetically intact rats, blockade of peripheral α_2-adrenoceptors with terazosin profoundly reduced the flare induced by CAP injection, whereas blockade of peripheral α_2-adrenoceptors by yohimbine did not obviously affect the flare. Therefore the pathogenesis of acute neurogenic inflammation in the intradermal CAP injection model depends in part on intact sympathetic efferents and α_2-adrenoceptors. Peripheral α_2-adrenoceptors thus modulate the ability of capsaicin sensitive afferents to evoke the release of inflammatory agents from primary afferents by DRRs.

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ceptor activation increases the secretion of nerve growth factor, a potent nociceptive peptide, from cultured vascular smooth muscle (Tuttle et al. 1993). Mechanical hyperalgesia produced by intradermal injection of prostaglandin E$_2$ was antagonized by phenolamine and prazosin (Ouseph and Levine 1995). It has been reported that the secondary hyperalgesia that follows intradermal injection of CAP could be blocked by intradermal injection of an $\alpha_1$-adrenoceptor antagonist into the CAP injection site (Kinnmann and Levine 1995a). These observations led us to determine if the ability of primary afferent fibers to evoke DRRs and the resulting neurogenic inflammation depends on peripheral sympathetic modulation of the excitability of CAP-sensitive afferent terminals.

This study was performed in an acute cutaneous neurogenic flare model that was induced by intradermal injection of CAP. We demonstrated in our previous study (Lin et al. 1999) that the spread of flare after CAP injection is mediated mainly by DRRs. Recently, a morphological study done by our group has shown that an increase in C-fos expression seen in spinal GABAergic neurons after CAP injection was reduced significantly after sympathectomy (Zou et al. 2002). Therefore we wanted to use this model to examine if sympathetic efferents modulate the DRR-mediated flare after CAP injection. Experiments were designed to determine if the spread of flare after CAP injection is eliminated or reduced by sympathectomy and the role of peripheral $\alpha$-adrenoceptors in the spread of flare in the foot skin after CAP injection.

Preliminary data have been published in abstract form (Lin et al. 2001).

**Methods**

Male Sprague-Dawley rats weighing 250–350 g were used for this study. Animals were initially anesthetized by sodium pentobarbital (50mg/kg ip) to perform surgery. The depth of anesthesia during surgery was adjusted by monitoring withdrawal responses to pinch. Anesthesia was then maintained during the experiment by intravenous infusion of sodium pentobarbital (5–8 mg·kg$^{-1}$·h$^{-1}$). Once a stable level of anesthesia was reached, the animals were paralyzed with pancuronium (0.3–0.4 mg/hr iv) and ventilated artificially. The level of anesthesia during the experiment was monitored by frequent ex-
amination of pupillary size, responses to stimulation, and stability of the level of end-tidal CO$_2$, which was kept between 3.5 and 4.5% by adjusting the respiratory parameters. Rectal temperature was moni-
tored using a rectal probe and maintained at 37°C adjusting the respiratory parameters. Rectal temperature was monitored using a rectal probe and maintained at 37°C.

All experimental protocols were approved by the Animal Care and Use Committee of the University of Texas Medical Branch and were in accordance with the guidelines of the National Institutes of Health and the International Association for the Study of Pain.

**Cutaneous blood flow measurements**

Blood flow was detected as blood cell flux by a laser Doppler flowmeter. The output showing blood flow level was then recorded by a computer data acquisition system (CED 1401 plus, with Spike-2 software) in millivoltage units (Fig. 2C). To measure the cutaneous blood flow level and the local vasodilation (flare) that followed intradermal injection of CAP into the skin of the foot, the probes from the laser Doppler flow meter (Moor Instruments) were attached to the plantar skin surface of the foot with adhesive tape. The flowmeter we used has been reported to produce a laser beam that penetrates to a depth of 500–700 $\mu$m below the surface where the probe is placed (Silverman et al. 1994). Therefore the laser Doppler flow probe presumably picked up the blood flow signal mainly from the micro-
vasculature in the dermis. As we have found in our previous work (Lin et al. 1999), the flare reaction after CAP injection could be detected at distances up to 30 mm away from the CAP injection spot. A large blood flow reaction was seen at a distance of 15–20 mm away from the site where CAP was injected, and this reaction has been demonstrated to be mainly mediated by DRRs (Lin et al. 1999). Therefore we measured the blood flow changes in the foot skin at a distance of 15–20 mm away from the CAP injection spot.

**Lumbar sympathectomy**

Surgical sympathectomy at the L$_{2-6}$ level was done as described by Kim et al. (1993). The sympathetic chains along with ganglia were identified through a transperitoneal approach. The exact levels were identified with the aid of a detailed description by Baron et al. (1988). All ganglia and the chains at L$_{2-6}$ were resected bilaterally. Animals were given postoperative care to allow for recovery from surgery for at least 1 wk before experiments were performed. A sham-operation was done on other animals as a control for the surgical procedure. At the termination of the experiment, the success of the sympathectomy was confirmed in each animal by examination of noradrenergic axons on the femoral artery on both sides with the fluorescent glyoxylic acid method (Furness and Costa 1975). Briefly, the animal was killed by an overdose of pentobarbital, and the femoral artery was dissected immediately. The dissected artery slip was split and immersed in 2% glyoxylic acid (pH 7.0, 0.1 M phosphate buffer) and incubated on a shaker for 30 min. Tissues were then mounted on glass slides after which they were air-dried and incubated for 4 min in a 100°C oven. Catecholamine-positive nerve fibers were examined under a fluores-
cent microscope (BP 395–440, FT 460 nm, LP 470 nm). All experi-
ments on sympathectomized rats were performed 7–10 days after sympathectomy and their absence was confirmed in sympathectomized rats.

**Decentralization of the sympathetic postganglionic neuron**

Possible involvement of activity in preganglionic sympathetic neu-
rons was examined by decentralizing sympathetic neurons prior to CAP injection. The sympathetic chain was visualized, and the sympa-
thetic trunk above the L$_{2}$ ganglia as well as white rami of the L$_{2}$ paravertebral ganglia were cut bilaterally. According to a detailed report by Baron et al. (1988), no white rami exist at the segments lower than L$_{3}$. Therefore resection of the sympathetic trunk above the L$_{2}$ ganglia and their rami would presumably cause a decentralization of almost all postganglionic neurons projecting to the hindlimb. CAP injections were performed at least 1 wk after decentralization. A histological examination showed that catecholamine-positive nerve fibers were present on the femoral artery after decentralization of the sympathetic postganglionic neurons (Fig. 1D).

**Peripheral administration of $\alpha$-adrenergic receptor agonists and antagonists**

One branch of the femoral artery on the side of blood flow mea-
surement was carefully isolated from connective tissue and ligated proximally. The artery was then cannulated distally by a small-sized polyethylene tubing that was connected with a Hamilton syringe. The $\alpha_1$- or $\alpha_2$-adrenoceptor agonists, phenylephrine (0.05 $\mu$g, Tocris) (Zarrindast and Sahebgharani 2002) or UK14,304 (0.3 $\mu$g, Tocris) (Buerkle and Yaksh 1998), were administered intra-arterially in a volume of 10 $\mu$l 10 min prior to CAP injection in sympathectomized rats. The $\alpha_1$- or $\alpha_2$-adrenoceptor antagonists, terazosin (10 $\mu$g, Sigma) or yohimbine (15 $\mu$g, Sigma), were administered locally by a bolus injection of 10 $\mu$l of solution into the artery 10 min prior to CAP.
injection in sympathetically intact rats. Terazosin has been reported to be a highly specific α1-receptor antagonist (Kyncl 1986), and it antagonizes the pressor response by phenylephrine at a dose of 10 μg given intracerebroventricularly (Yuki et al. 1987). Yohimbine produces a selectively antagonistic effect on the α2-receptor agonist-evoked antinociception at doses between 10 and 100 μg given intrathecally (Howe et al. 1983).

In other rats, the vehicle (saline) that was used to dissolve the drugs was injected intra-arterially at the same volume as a control.

**Experimental protocol**

To evoke an acute flare reaction, CAP, dissolved in Tween 80 (7%) and saline (93%) to a concentration of 1% with a volume of 15 μL, was injected intradermally into the foot skin 15–20 mm away from the site where the blood flow was recorded. Blood flow on the plantar skin of the foot was first recorded both in groups of sympathectomized and sham-sympathectomized rats before and after intradermal injection of CAP on the same side where blood flow was measured. To determine if the CAP injection itself could produce systemic effects, such as a change in blood flow due to a change in systemic blood pressure, blood flow changes in the plantar skin of a forepaw were also recorded simultaneously. The third group included the rats with decentralized sympathetic postganglionic neurons. Changes in blood flow after CAP injection were recorded in the same fashion as described in the preceding text.

To determine further the involvement of peripheral sympathetic outflow in the CAP-induced flare, the following manipulations were performed. 1) Observations were made on the effects of activation of peripheral α-adrenoceptors on the CAP-induced flare produced under sympathetically intact conditions. In one group of sympathectomized rats, after control blood flow level was recorded for 30 min, the α1- or α2-adrenoceptor agonists, phenylephrine (0.05 μg) or UK14,304 (0.3 μg), were administered intra-arterially in a volume of 10 μL 10 min prior to CAP injection. Changes in blood flow after CAP injection were then recorded for 1.5–2 h. A control experiment was done by intra-arterial injection of the vasoconstrictor, vasopressin (0.15 μg), in a different group of sympathectomized rats. 2) Intra-arterial injection of terazosin (10 μg) or yohimbine (15 μg) was done under sympathetically intact conditions to examine if blockade of α1- or α2-adrenoceptors could affect the CAP-induced flare. In one group of sympathetically intact rats, terazosin or yohimbine was injected intra-arterially 10 min before CAP was injected intradermally. Changes in blood flow after CAP injection were then recorded for 1–1.5 h. As controls, saline, the vehicle used for dissolving drugs, was also injected intra-arterially prior to CAP injection in a different group of rats.

In our previous study, we demonstrated that elimination of DRRs by blockade of spinal cord GABAγ, N-methyl-D-aspartate (NMDA), or non-NMDA receptors can profoundly reduce the widespread flare induced by CAP injection (Lin et al. 1999). Here we wanted to test if activation of α-adrenoceptors could still affect the CAP-induced flare produced under sympathetically intact conditions after DRRs were reduced by blocking spinal GABAγ receptors. In sympathectomized rats, a GABAγ receptor antagonist, bicuculline (5 μg, dissolved in 15 μl artificial cerebrospinal fluid, ACSF), was injected intrathecally 20 min prior to CAP injection as described previously (Lin et al. 1999). Phenylephrine was then injected intra-arterially in the same dose as mentioned in the preceding text 10 min before CAP injection. Changes in blood flow after CAP injection were then recorded for 1–1.5 h. A control experiment was done in which intrathecal injections of ACSF were made in a different group of sympathectomized rats.

**Data analysis**

Baseline blood flow level was expressed as 100% and percentage changes after CAP injection were compared for different groups of animals. Statistical significance was tested using ANOVA with repeated measures, and differences across time were assessed with paired t-tests. A grouped t-test was used to compare the difference in responses between groups having different treatments. $P < 0.05$ was taken as significant. Values are expressed as means ± SE.
RESULTS

Changes in cutaneous blood flow from the ipsilateral foot after capsaicin injection and effects of sympathectomy and sympathetic decentralization

Observations on the blood flow reaction to intradermal CAP injection were made in two groups of rats. One group was sham-sympathectomized rats. These rats underwent sham surgery without removing the lumbar sympathetic chains and ganglia. Consistent with our previous report (Lin et al. 1999), an elevated blood flow was seen at both sites at which blood flow was measured on the foot (probes 1 and 2) after CAP injection in the sham-sympathectomized rats. Probe 1 was placed near the CAP injection site, and probe 2 was 15–20 mm away from the site of CAP injection. The enhanced responses recorded by probe 1 (Fig. 2A) were less than the responses measured by probe 2 (Fig. 2A). Peak increases were 247.4 ± 50.9% (P = 0.002, compared with baseline level) recorded from probe 1 and 457.3 ± 51.6% (P = 0.001) recorded from probe 2. In the sympathectomized group of rats, the enhanced blood flow recorded from both sites (Fig. 2A) was less than in rats with sham surgery, but the magnitude of the reduction in the response at probe 2 was much larger than that near the CAP injection site (probe 1). Peak increases were 233.4 ± 10.3% (P = 0.017) recorded from probe 2 and 190.9 ± 20.8% (P = 0.027) recorded from probe 1. The peak increase and the value at 60 min after CAP injection recorded from probe 2 in the sympathectomized group became much smaller than that in the sham-operated group (P = 0.006 and P = 0.002, Table 1). There was no statistical difference in the peak increases measured by the probe 1 between the sympathectomized and the sham-operated groups (P = 0.317, Table 1), but the blood flow level at 60 min after CAP injection in sympathectomized rats was significantly lower than that in sham-operated rats (P = 0.02, Table 1). Thus the blood flow reaction recorded both from probes 1 and 2 induced by CAP injection recovered sooner after sympathetic efferents were removed. Examples of the laser Doppler blood flow recordings at the probe 2 site are shown in Fig. 2C for both sham-operated and sympathectomized rats.

A previous study done in the same model showed that an intradermal vehicle (Tween 80 and saline) injection did not
produce obvious changes in blood flow in the foot skin (Lin et al. 1999).

To exclude the possibility that the blood flow reaction recorded locally from the paw skin after an ipsilateral CAP injection was the result of changes in systemic blood pressure, the blood flow in the skin of the forepaw was recorded simultaneously both in sympathectomized and sham-sympathectomized rats. A slight increase in blood flow was seen right after CAP injection, but this was not statistically significant (P = 0.249 for sympathectomized, P = 0.232 for sham-sympathectomized, Fig. 2B).

The concern arises as to whether the cutaneous bed was dilated in the absence of constrictor tone after sympathectomy, which might prevent the cutaneous bed from dilating much further after CAP injection. Figure 2C shows the resting blood flow level in the skin of the hindpaw ipsilateral to CAP injection and its change after CAP injection both in a sham-operated and a sympathectomized rat, and Fig. 2D is a summary of resting blood flow level in both groups. There was no significant change in resting blood flow level after sympathectomy compared with the sham-operated group.

CAP injection still gave rise to a remarkable increase in blood flow response to CAP injection after transection of the preganglionic efferents (Fig. 2A, decentralization sympathectomized group). The increased blood flow reaction after CAP injection seemed to last even longer than that seen in the sham-sympathectomized rats (Fig. 2A), but no significant difference was found. Thus preganglionic sympathetic activity is not necessary for the development of flare induced by CAP.

Effects of activation of peripheral α-adrenoceptors on blood flow responses after capsaicin injection under sympathectomized conditions

Under sympathectomized conditions, peripheral α1- or α2-adrenoceptors were activated by intra-arterial injection of phenylephrine or UK14,304 10 min prior to intradermal CAP injection. A decrease in blood flow level was seen both at probe 1 and 2 sites immediately after either phenylephrine or UK14,304 was injected intra-arterially (Fig. 3). After this decrease, there was a large increase in blood flow immediately after CAP injection at the probe 2 site after phenylephrine administration, but not after UK14,304 (Fig. 3B). The peak increase with phenylephrine pretreatment was to 360.8 ± 14.3%. This enhancement was comparable to the increase in blood flow seen under sham-sympathectomized conditions (Table 1). The enhanced blood flow could last up to 2 h (325.9 ± 30.5%) after CAP injection (Fig. 3B). However, there were no significant changes in blood flow at the probe 1 site after CAP injection at the probe 2 site after phenylephrine administration, but not after UK14,304 (Fig. 3B). The peak increase with phenylephrine pretreatment was to 360.8 ± 14.3%. This enhancement was comparable to the increase in blood flow seen under sham-sympathectomized conditions (Table 1). The enhanced blood flow could last up to 2 h (325.9 ± 30.5%) after CAP injection (Fig. 3B). However, there were no significant changes in blood flow at the probe 1 site

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<th>TABLE 1. Peak increases and values after CAP injection</th>
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P values are from comparisons between the sham-sympathectomized and sympathectomized groups and between sham-sympathectomized and drug treatment groups. Reduction in the flare at probes 1 and 2 on the foot induced by intradermal injection of capsaicin (CAP) after sympathectomy (SYMP) and the effects of local injections of α1- and α2-adrenoceptor agonists or vasopressin. The drugs tested were the α1-adrenoceptor agonist, phenylephrine (Phenyl), the α2-adrenoceptor agonist, UK14,304, and vasopressin (VP).
phenylephrine pretreatment did not produce much of an enhancement after phenylephrine was given. As shown in Fig. 4, phenylephrine, and CAP was injected intradermally 10 min prior to intra-arterial administration with ACSF (410.1 ± 29.1%; Fig. 3A, Table 1). In contrast, pretreatment with UK14,304 by intra-arterial injection did not significantly change the blood flow response induced by CAP injection (Fig. 3, Table 1). To exclude the possibility that the increase in blood flow after phenylephrine and CAP injections was secondary to vasoconstriction, a control experiment was done in which the paw was pretreated with vasopressin. Vasopressin did not affect the blood flow changes after CAP injection (Fig. 3B).

In sympathectomized rats, we have further examined if activation of α₁-receptors could still restore the CAP-evoked flare after DRRs were greatly reduced by blockade of spinal GABA_A receptors. The spinal cord was pretreated with bicuculline intrathecally 10 min prior to intra-arterial administration of phenylephrine, and CAP was injected intradermally 10 min after phenylephrine was given. As shown in Fig. 4, phenylephrine pretreatment did not produce much of an enhancement of the CAP-evoked flare at probe 2 after spinal GABA_A receptors were blocked by bicuculline. The peak increase was 158.2 ± 8.9% (P = 0.002, compared with baseline level) at probe 2. This was a much smaller increase compared with the peak increase in the group of sympathectomized rats pretreated with ACSF (410.1 ± 42.7%, P = 0.0003). Bicuculline pretreatment only slightly reduced the flare at probe 1 (peak increase was 211.6 ± 13.1%, P = 0.031, compared with baseline level) induced by CAP combined with phenylephrine pretreatment when compared with the flare at the same location in the group of sympathectomized rats that were pretreated with ACSF (peak increase was 298.5 ± 56.4%, P = 0.012, compared with baseline value), but the reduction did not reach statistical significance when a comparison was made of the peak values in these two groups (P = 0.264).

These experiments are consistent with the interpretation that sympathetic efferents are involved in the modulation of CAP-evoked flare by activation of peripheral α₁-receptors and that the flare is mediated by way of DRRs.

**Effects of blockade of peripheral α-adrenoceptors on blood flow responses after capsaicin injection under sympathetically intact conditions**

In sympathetically intact rats, we have further examined if the blockade of α-adrenoceptors affected the CAP-induced flare. Because activation of either peripheral α₁- or α₂-adrenoceptors did not change significantly the CAP-evoked vasodilation at the probe 1 site under sympathectomized conditions, the observations on the effects of α₁- or α₂-adrenoceptor antagonists on the CAP-evoked vasodilation were only made at the probe 2 site. The antagonist was injected intra-arterially 10 min prior to CAP injection, and there was no obvious change in blood flow after drug injection. However, the flare induced at the probe 2 site by CAP injection was reduced dramatically after α₁-adrenoceptors were blocked by intra-arterial injection of terazosin (Fig. 5). The peak increase was 154.9 ± 13.0%, which was significantly lower than when the paw was pretreated with saline (peak increase 411.5 ± 26.7%, Table 2). In contrast, blockade of α₂-adrenoceptors by intra-arterial injection of yohimbine did not significantly affect the flare reaction after CAP injection. The peak increase was 373.1 ± 41.1%, which was comparable to the increase in blood flow in the saline-treated group (Fig. 5, Table 2).

**DISCUSSION**

Sympathectomy or sympathetic block is effective in reducing pain behaviors in some neuropathic and inflammatory pain models (Kim and Chung 1991; Kinnmann and Levine 1995b; Levine et al. 1986; Moon et al. 1999; Neil et al. 1991; Xie et al. 1995). In the present study, we have used the acute cutaneous inflammation that results from intradermal injection of CAP to study the sympathetic modulation of neurogenic inflammation. The inflammatory model we have used is characterized by vasodilation both near the CAP injection site and in a surrounding area that extended more than 30 mm away from the injection site. In our previous study, we demonstrated that the spread of flare in rats is mediated by DRRs (Lin et al. 1999). Here we have shown that sympathectomy resulted in a dramatic decrease in the DRR-mediated flare measured at the distant site (probe 2) after CAP injection. However, if peripheral α₁-adrenoceptors were activated prior to CAP injection under sympathectomized conditions, the flare induced by CAP injection could be restored. Under sympathetically intact conditions, blockade of peripheral α₁-adrenoceptors could prevent the CAP-evoked flare. Thus the spread of flare mediated by DRRs following CAP injection seems to be sympathetically dependent, and peripheral α₁-adrenoceptors play a role in this process.
Our group has developed models of neurogenic inflammation both in knee joint and skin (Lin et al. 1999; Sluka and Westlund 1993). One of the mechanisms underlying the inflammation in these models involves spinal mediation antinociceptive and sympathetic activity in primary afferent fibers (DRRs) (Lin et al. 1999, 2000b; Rees et al. 1994; Sluka et al. 1993, 1995a). Based on experimental evidence obtained from these models, we have proposed that neurogenic inflammation is produced in following way (Sluka et al. 1995b; Willis 1999; Willis et al. 1998, 2000). CAP injection activates C and some Aδ nociceptors. This enhanced afferent discharge activates GABAergic interneurons in the spinal dorsal horn by release of glutamate onto non-NMDA and NMDA receptors (Zou et al. 2001). The GABAergic interneurons in turn release GABA, which acts on GABA_A receptors to produce primary afferent depolarization (PAD). CAP injection results in an increased afferent activity that drives these interneurons, so their excitability is increased and they produce a larger PAD in the nociceptive afferents, which, in turn, generate DRRs. The DRRs travel antidromically to the periphery and evoke flare and edema by release of inflammatory substances, including CGRP and SP.

However, neurogenic inflammation depends not only on the excitation of the primary afferent terminals but also on the presence of sympathetic postganglionic neurons (Heller et al. 1994). Peripheral injury results in plastic changes of both afferent and sympathetic postganglionic neurons, leading to chemical coupling between sympathetic and afferent neurons (see reviews by Heller et al. 1994; Jäning et al. 1996). The activation of peripheral terminals of the sympathetic postganglionic neurons has been shown to be an important contributor to neurogenic inflammation. Sympathetic efferents affect the release of inflammatory agents from primary afferent terminals by releasing NE and/or non-adrenergic substances, such as prostaglandins, purines and neuropeptide Y. Sympathectomy significantly reduces plasma extravasation induced either by CAP or bradykinin injection (Bjerknes et al. 1991; Codere et al. 1989; Miao et al. 1996a,b). Plasma extravasation induced by SP, histamine, or bradykinin can also be reduced by sympathectomy (Gonzales et al. 1991; Khalil and Helme 1989).

In our present study, evidence that the spread of flare after CAP injection was profoundly reduced after postganglionic sympathetic efferents were removed surgically supports strongly the view that the generation and development of neurogenic inflammation depends on intact postganglionic sympathetic efferents. NE is presumed to be a mediator in establishing a pathological coupling between the primary afferents and postganglionic sympathetic efferents (Jäning et al. 1996; Michaelis 2000). Several studies have reported that either stimulation of sympathetic efferents or local application of NE can excite primary nociceptors under the conditions of tissue inflammation or nerve injury (Nam et al. 2000; O’Halloran and Perl 1997; Sato and Kumazawa 1996; Sato and Perl 1991; Sato et al. 1993). The number of α-adrenergic receptors in dorsal root ganglion cells increases markedly after sciatic nerve injury (Birder and Perl 1999). However, an unresolved issue is whether NE sensitizes nociceptors directly or indirectly. A study by Levine’s group (Kinnmann and Levine 1995a) showed that the secondary hyperalgesia after intradermal injection of CAP could be blocked by intradermal injection of an α1-adrenoceptor antagonist into the CAP injection site. These observations led to the view that catecholamines do not directly sensitize primary afferents but act on α-receptors either on nearby postganglionic fibers or on primary afferent terminals, which probably causes release of other compounds that may subsequently mediate hyperalgesia. On the other hand, it has also been reported that intact unmethylated cutaneous nociceptors became sensitive to NE after adjacent nerves were injured (Ali et al. 1999; Koltzenburg et al. 1994; Sato and Perl 1991).

Our experiments have shown that local activation of α1- adrenoceptors, but not α2-adrenoceptors, can restore the spread of flare induced by CAP injection. Because the peripheral tissue has been sympathetically denervated, we presume that the α1 receptors activated should be located on the primary afferent terminals and that the CAP-evoked vasodilation is normally dependent on the presence of postganglionic sympathetic efferents, which release NE to modulate the responses of the nociceptors to CAP by acting on α1 receptors. As shown in the present study, local administration of either α1- or α2-
adrenoceptor agonists produced vasoconstriction. However, activation of α1-adrenoceptors helped produce the flare reaction induced by CAP injection, suggesting that α1 receptors help maintain the sensitivity of nociceptor terminals to CAP. The control experiments using local injection of vasopressin exclude the possibility that vasoconstriction itself produces vasodilatation secondarily. The fact that modulation of primary afferent terminals by sympathetic postganglionic efferents is independent of preganglionic efferent activity is consistent with the results of a behavioral study (Kinnmann and Levine 1995a). We also show that blockade of peripheral α1-adrenoceptors with terazosin in sympathetically intact rats dramatically reduced the spread of flare induced by CAP injection, suggesting that there is an endogenous release of NE from postganglionic sympathetic efferent terminals.

The vasodilation near the site of CAP injection results mainly from local axon reflexes or from a direct action of CAP on sensory terminals (Szolcsanyi 1996). Consistent with this, we have found that neither sympathectomy nor local injection of phenylephrine affected significantly the vasodilatation recorded from the site near the CAP injection spot (probe 1).

To conclude, the acute cutaneous neurogenic inflammation produced by intradermal CAP injection has been demonstrated to be triggered by centrally mediated antidromic activity in primary afferent nociceptors. The present data suggest further that this pathophysiological process depends on intact postganglionic sympathetic efferents. Release of NE appears to activate α1-adrenergic receptors, which are presumably located on the primary afferent terminals. This enhances the responses of the afferent terminals of nociceptors to CAP. The central effects of the CAP-evoked afferent activity include antidromic activity, which in turn triggers the release of inflammatory agents from primary afferent terminals.

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