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Involvement of Alpha1-adrenoceptors in the Cutaneous Blood Flow Increase Response to Sympathetic Nerve Stimulation in Rats with Chronic Constriction Injury

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Abstract: There is some evidence that the sympathetic nervous system plays a role in the development and/or maintenance of painful states, and that sympathetic nervous function is altered in these conditions. Our previous experiments showed that electrical stimulation of the lumbar sympathetic trunk (sympathetic stimulation: SS), which normally induces a decrease in blood flow (BF), induced a BF increase in all adjuvant-inflamed rats. Such rats are one model of persistent pain. This mechanism for the BF-increase response to SS involves both α-adrenergic and non-adrenergic receptors. In the present experiment we examined whether BF was increased by SS in another animal model of pain, which was produced by placing loosely tied ligatures around the common sciatic nerve, and whether the BF response involved the α1-adrenoceptor. We found SS induced both increased and decreased BF responses in this pain model. Both responses were inhibited by α1-adrenoceptor antagonist. This result suggests the possibility that the plantar BF-increase response occurs as a secondary effect following vasoconstriction at another site.

Key words: sympathetic nerve stimulation, neuropathic pain, blood flow, rats

Introduction

Sympathectomy, or sympathetic ganglion blockade, has been shown to reduce pain in some chronic pain conditions such as complex regional pain syndrome (CRPS) (Butler 1990; Schwartzman 1992; Scadding, 1999). Abnormalities of the skin temperature, coloring of the affected areas and sweating have been observed in this condition (Scadding, 1999). These clinical findings suggest sympathetic nerve activity plays important roles in the development and/or maintenance of pain and accompanying changes of the affected tissues in these pathological conditions.

On the other hand, sympathetic involvement in these tissue changes has been less intensively investigated. Wakisaka reported that both clinical and experimental neuropathic conditions are accompanied by an abnormality of cutaneous temperature regulation in the painful area (Wakisaka et al, 1991). In our previous experiment investigating the involvement of sympathetic nervous activities in tissue change in painful conditions, we found that electrical stimulation of lumbar sympathetic nerve trunk (sympathetic stimulation: SS), which generally produces vasoconstriction of cutaneous blood vessels in normal animals, induced an increase in blood flow (BF) in the plantar skin of rats with adjuvant-induced inflammation (AI) (Koeda et al, 2002). This altered vascular response involved both α-adrenergic and non-adrenergic mechanisms. It is possible that vascular response is also altered in neuropathic pain models such as the chronic constriction injury (CCI) model (Bennett and Xie, 1988), although this has not been proven.

One of the aims of the present study, therefore, is to examine whether the vascular response to SS is altered, i.e., a vasodilating response, in CCI rats. The second is to examine whether α1-adrenoceptors, similar to their action in inflammation models, are responsible for this effect of SS.

Materials and Methods

1. Animals:

Fourteen male Sprague-Dawley rats (SLC Inc., Japan) were used. Nine rats were subjected to painful mononeuropathy under pentobarbital sodium anesthesia (50 mg/kg, i.p.) by 4 loose ligatures of chromic gut around the right sciatic nerve (Bennett and Xie, 1988). The sciatic nerves of other rats were exposed, but not ligated. All rats were kept on a 12-h light/dark cycle at 23°C with free access to food and water. All experimental procedures were approved by the Animal Care Committee, Research Institute of Environmental Medicine,

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2. Experimental protocols:

Rats were used 4 days after surgery. The temperature of the middle plantar surface of both hind paws was measured with rats in the prone position before surgical operation under α-chloralose and urethane anesthesia (90 and 450 mg/kg i.p., respectively). Accuracy of the temperature measurement was ± 0.1°C.

Methods of surgery, lumbar sympathetic trunk stimulation, and laser Doppler flowmetry were previously described in detail (Koeda et al, 2002). Briefly, the right carotid artery was cannulated to measure the mean arterial pressure (MAP). A branch of the femoral artery was cannulated for drug administration. The lumbar sympathetic trunk (LST) was sectioned between L3 and L4 ganglia and communicating branches of spinal nerves in the L4 ganglion were cut. The LST was kept in paraffin oil, and then its peripheral cut end was placed on a bipolar platinum-stimulating electrode. The LST was stimulated by rectangular pulses of 0.2 ms duration and 10 V strength, at 1~5 Hz for 10 sec. The frequency of the SS was adjusted so that the greatest vasodilating response was obtained; 3 Hz was the most frequently used. The effects of SS were tested at intervals longer than 10 min. When supplemental anesthetic drug was added, a minimum of 10 min elapsed before the next SS.

The relative change in local BF was measured in the plantar skin of the right hind paw with a laser Doppler flowmeter (ALF21, Advance Co., Ltd., Tokyo, Japan). BF was simultaneously measured at two sites. Electrical signals from the laser Doppler flowmeter and blood pressure monitor were simultaneously recorded and fed into a computer through an AD converter (DIGIDATA 1200, Axon Instruments, Inc., Foster City, CA, USA) using a data analysis program (AXOTAPE, Version 2.0, Axon Instruments, Inc., Foster City, CA, USA). The sampling intervals of the AD converter were 40 ms in the SS experiment and 100 ms with the administration of drugs. The sampled data were averaged every 1 sec and expressed as arbitrary units for the BF and mmHg for the MAP. Apparent vascular conductance (VC) was calculated as the ratio of the BF to the MAP. The BF, MAP and VC values over 60 s before stimulation or drug administration were averaged and used as the baseline values. A change in the BF exceeding the baseline by more than twice the standard deviation of the baseline value was used as the criterion for a response. To quantitatively analyze vascular changes, the difference between the baseline value and the maximum (or minimum) value after SS was calculated (as shown by ΔBF).

3. Drug administration:

The effects of prazosin (Sigma, St. Louis, MO, USA), an α1-adrenoceptor antagonist were examined. The drug was dissolved in distilled water and the infused volume was 30 μl. Saline was infused before and after administration of drugs without interruption since the BF was affected by the infusing fluid itself.

4. Statistical analysis:

The statistical significance was determined as follows: One way analysis of variance (ANOVA) with repeated measures followed by Bonferroni's test was used for comparison of the blood flow (BF) and mean arterial pressure (MAP) changes after drug administrations, and two-tailed Wilcoxon's test for comparisons of the BF change after SS in the presence as well as the absence of antagonists. Results were considered significant if $p<0.05$.

Results

Baseline hind paw temperatures ranged from 25.5 to 33.6°C on the operated side and from 25.4 to 30.2°C on the non-operated side (n=7). The temperature differences between the left and right hind paws of individual rats are shown in Fig. 1. The temperature difference (operated side minus non-operated side) was 1.3 ± 1.6°C in CCI rats, against –0.5 ± 1.5°C in controls; thus, it was significantly higher in CCI rats ($p<0.05$).

SS induced a decrease in BF in all normal rats, while it induced either an increase or a decrease in BF in the CCI rats. Typical BF responses to SS in the CCI rats are shown in Fig. 2A and C. In this case two probes of the laser Doppler flowmeter were placed on the plantar skin, and the BF responses to SS at these two sites were recorded simultaneously. To determine whether there was any area with a preferential BF-increase response in other cases, we trisected the plantar skin from the heel to the base of the toes and measured the BF

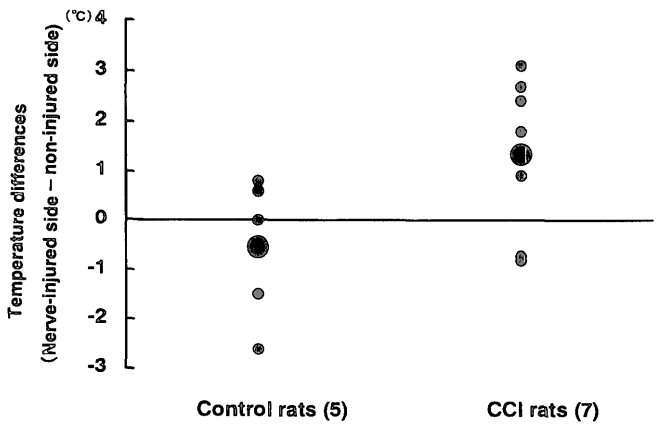

Fig. 1 Temperature differences of the paw in the control and CCI rats. Ordinate: Difference in the temperature of the injured side from the non-injured side. Each small circle denotes a different rat. Larger circles show the means. Number of rats is shown in parenthesis.

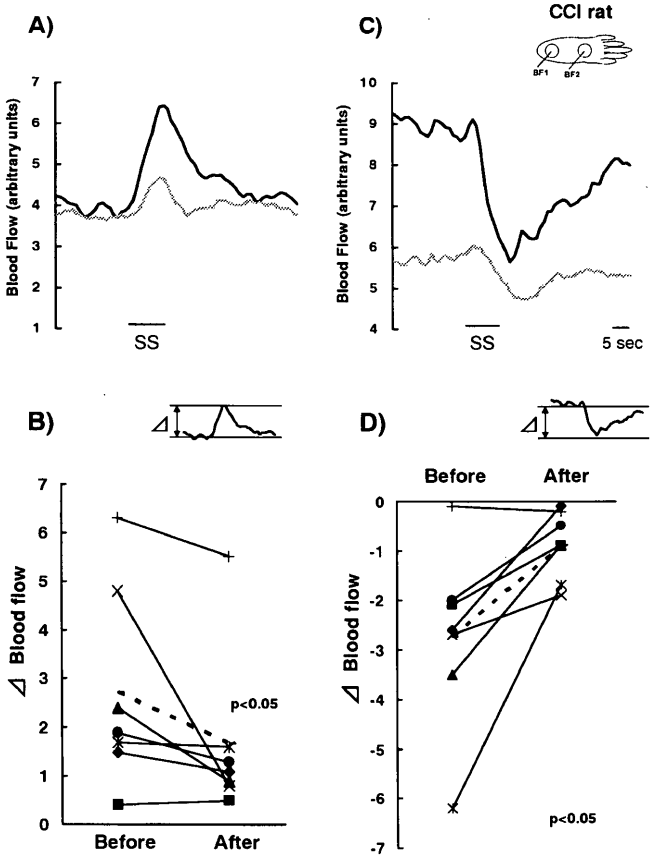

Fig. 2 Effects of alpha1-adrenoceptor antagonist on the BF responses to SS in CCI rats (n=7).
A), C): Sample recordings. Solid line, before administration of prazosin (0.03 mg/kg); dotted line, after administration of prazosin in the same CCI rat. Ordinate: BF in arbitrary units. Bar: period of SS. B), D): Summary of the effect of prazosin. Ordinate: changes in BF from baseline value (DBF). Either the maximum (BF-increase response) or the minimum (BF-decrease response) values after SS were measured as shown in the insets. Each symbol denotes a different rat. Dotted line is the mean. Prazosin significantly inhibited both the BF-increase and -decrease responses to SS ($p<0.05$).

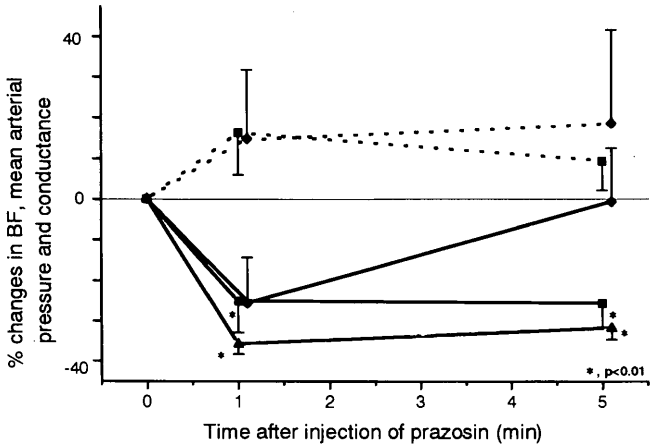

Fig. 3 Changes in basal BF, MAP, and conductance induced by prazosin (n=7).
Ordinate: % changes in BF, MAP, and conductance from the level before administration of prazosin. Rhombuses represent vascular changes in the BF-increase site, and squares those in the BF-decrease site. Triangles represent MAP. Solid lines are BF and dotted lines are conductance. MAP and BF at the site where BF decreased in response to SS significantly decreased after administration of prazosin ($p<0.01$).

at only one site randomly selected in each area. SS decreased the BF in all sites in the control group (n=5). In contrast, all CCI rats (n=6) had BF-increase responses in more than one area measured. The percentage of randomly sampled sites exhibiting the BF increase response was 33.3% at the heel, 50.0% in the middle of the sole and 33.3% at the base of the toes in the CCI rats. There was no significant difference in these ratios among the three areas (χ-square test, $p>0.05$).

We examined the effects of prazosin, an α1-adrenoceptor antagonist, on SS-induced BF responses in the CCI rats (n=7). Figure 2 shows a representative recording and summary of the effects of prazosin on the BF responses to SS. The BF-decrease response to SS was significantly reduced by prazosin. It also significantly suppressed the SS-induced BF increase response ($p<0.05$, Fig. 2). Prazosin (0.03 mg/kg) significantly decreased the baseline BF at the sites where BF decreased in response to SS, and thereafter the BF remained at this decreased level (Fig. 3, $p<0.01$). Prazosin did not significantly influence the baseline BF at the sites where the BF increased in response to SS. MAP decreased significantly and then stabilized at a somewhat lowered level 5 min after injection of prazosin ($p<0.01$). This MAP change suggests that the observed BF decrease was mainly due to the decreased perfusion pressure, rather than that α1-adrenoceptor exerted a vasodilating action.

Discussion

In the present study, the skin temperature on the operated side was higher than on the non-operated side in almost all CCI rats 4 days after surgery. This result is in accordance with an earlier report (Wakisaka et al, 1991).

We found, in addition, that SS simultaneously induced both the BF-increase and -decrease responses in the same CCI rat. Sites of BF increase in response to SS were scattered over all areas of the sole of the hind paw. This abnormal vascular response was also seen in AI rats (Koeda et al, 2002). Clinical observation showed that the persistent pain state is accompanied by an abnormality of the sympathetic nervous system. We have shown that both inflammation and neuropathy models are also accompanied by an abnormality of the adrenergic vasomotor innervations of the symptomatic hind paw.

Both BF-increase and -decrease responses to SS were suppressed by α1-adrenoceptor antagonist. It is difficult to conclude that this BF-increase response to SS was a direct effect, since these mirrored responses were suppressed by the same antagonist. The BF-increase responses to SS observed in this

experiment would have been passive responses secondary to vasoconstriction in other areas. Reduction by prazosin of the SS-induced BF-increase and -decrease response supports this passive mechanism hypothesis. This mechanism might require fewer α1-adrenoceptors at the sites of BF increase than at those of BF decrease. It is reported that at 5 days after nerve injury, the distribution and number of noradrenaline (NA)-positive axons on the plantar vessels were unmistakably or very markedly decreased on the nerve-injured side (Wakisaka et al, 1991). This loss of NA vasomotor fibers might be not evenly distributed. The basal BF did not significantly decrease at the sites where BF increased in response to SS after administration of prazosin, although the basal BF significantly decreased at the BF-decrease response sites. This also supports the supposition of adrenergic vasomotor fiber loss at the BF-increase response site. These results suggest that BF-increase response to SS occurs secondary to vascular constriction at other sites.

References

Bennett GJ and Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87–107.

Butler SH. Reflex sympathetic dystrophy: Clinical features. In: Stanton-Hicks M, Janig W, eds. Reflex sympathetic dystrophy. Boston: 1990: 1–8.

Koeda T, Sato J, Kumazawa,T, et al. Effects of adrenoceptor antagonists on the cutaneous blood flow increase response to sympathetic nerve stimulation in rats with persistent inflammation, Jpn J Physiol 2002; 52: 521–530.

Scadding JW. Complex regional pain syndrome. In: Wall PD and Melzack RM, eds. Textbook of pain. London: Churchhill Livingstone, 1999: 835–849.

Schwartzman RJ. Reflex sympathetic dystrophy and causalgia, Neurol Clin 1992; 10: 953–973.

Wakisaka S, Kajander KC, and Bennett GJ. Abnormal skin temperature and abnormal sympathetic vasomotor innervation in an experimental painful peripheral neuropathy, Pain 1991; 46: 299–313.

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