Responses of Dorsal Spinal Cord Blood Flow to Noxious Mechanical Stimulation of the Skin in Anesthetized Rats

Hiroko TODA¹, Hitoshi MARUYAMA², Brian BUDGELL³, and Mieko KUROSAWA¹,⁴
¹Center for Medical Science, International University of Health and Welfare, Otawara, Tochigi, 324-8501 Japan; ²Department of Physiotherapy, International University of Health and Welfare, Otawara, Tochigi, 324-8501 Japan; ³Département de Chiropratique, Université du Québec à Trois-Rivières, Canada; and ⁴School of Pharmacy, International University of Health and Welfare, Otawara, Tochigi, 324-8501 Japan

Abstract: In urethane-anesthetized, artificially ventilated rats, alterations in dorsal spinal cord blood flow (SCBF) at the L4–6 level were measured with laser Doppler flowmetry in response to noxious mechanical cutaneous stimulation (pinching) of either a forepaw or a hindpaw. The stimulation was delivered ipsilaterally or contralaterally to the site of blood flow measurement. Pinching of the forepaw or the hindpaw on either side increased mean arterial pressure (MAP) to the same degree. However, the SCBF response to pinching of the ipsilateral hindpaw was significantly greater than that to other stimulations. These responses were not influenced by denervation of the baroreceptors. The responses of SCBF to pinching of the ipsilateral hindpaw persisted both after treatment with phenoxybenzamine and after spinalization at the C1–2 level, whereas the responses to pinching at other sites disappeared. The responses of MAP to stimulation at all four sites became negligible after treatment with phenoxybenzamine and after spinalization at the C1–2 level. These results indicate that noxious mechanical stimulation of the skin produces increases in SCBF via two mechanisms: one is via an elevation of systemic arterial pressure; the other is via a localized spinal mechanism evoked by ipsilateral, segmental inputs.

Key words: spinal cord blood flow, noxious mechanical stimulation, pinching, blood pressure, segmental organization.

The physiology of the spinal cord is dependent upon appropriate blood flow, and impaired spinal cord blood flow (SCBF) leads to spinal dysfunction [1, 2]. It has recently been shown, in anesthetized rats, that innocuous mechanical stimulation (brushing) of the skin increases dorsal SCBF without any change in systemic arterial pressure. The increases in SCBF were observed when brushing was applied to dermatomes ipsilateral to and at the same segmental level as the site of SCBF measurement [3]. Furthermore, it has been demonstrated that the increases in SCBF persist after spinal cord transection [4].

Autonomic responses to somatic afferent stimulation differ depending on whether the stimulus is noxious or innocuous. For example, noxious pinching increases systemic arterial blood pressure whereas innocuous brushing has no such influence [5]. Also, adrenal catecholamine secretion increases in response to noxious pinching stimulation whereas it decreases in response to innocuous brushing stimulation [6]. Since pinching produces increases in systemic arterial blood pressure [7], and, in turn, increases in systemic arterial blood pressure have been shown to enhance regional blood flow in other organ systems [5, 8], the SCBF responses to pinching may differ from responses to innocuous brushing despite autoregulation.

Therefore, the present study determined the responses of SCBF to pinching of the skin in anesthetized rats. The changes in dorsal SCBF were measured at the level of L4–6, which receives afferent inputs from the hindlimb and hindpaw, and pinching was applied to the ipsilateral or contralateral hindpaw (segmental stimulation) or forepaw (extra-segmental stimulation). In order to clarify the contribution of systemic arterial blood pressure to the SCBF responses, experiments were also conducted in baroreceptor-denervated animals, in animals in which systemic arterial blood pressure responses were blocked with phenoxybenzamine. Furthermore, experiments with spinal cord transection were conducted in order to resolve spinal and supraspinal contributions to the SCBF responses.

MATERIALS AND METHODS

Animal care and housing. Experiments were performed on 24 male Wistar rats (350–430 g). The animals were
kept in a temperature-controlled room (23 ± 1°C) that was illuminated between 08:00 h and 20:00 h. Commercial rodent chow (Labo-MR stock, Nosan Corporation, Kanagawa, Japan) and tap water were provided ad libitum. This study was approved by the animal ethics committee of the International University of Health and Welfare.

General experimental procedures. The rats were anaesthetized with an intraperitoneal injection of urethane (1.1 g·kg⁻¹). The animals were intubated, and ventilation was maintained artificially (Model SN-480-7 ventilator, Shinano, Tokyo, Japan). The right jugular vein was cannulated for the administration of supplemental anaesthetic and other substances according to the experimental protocol. Blood pressure was monitored continuously from the right carotid artery. Core temperature was maintained at 37.0 ± 0.1°C by a heating pad and an infrared lamp (ATB-1100, Nihon Kohden, Tokyo). Throughout the experiment, the depth of anaesthesia was routinely judged by observing the fluctuation in blood pressure.

Cutaneous stimulation. Noxious mechanical stimulation was performed by pinching the forepaw or the hindpaw unilaterally (approximately 1 cm²) with a surgical clamp (about 3–5 kg force) for 30 s. Stimuli were applied 2–4 times per rat, and data from identical procedures were pooled to produce an averaged response for each animal.

Measurement of SCBF. Laminctomy was performed at T13–L1 to expose the dorsal surface of the L4–6 segments of the spinal cord. The probe (Type N, 0.5 mm diameter, Advance Co., Tokyo, Japan) of the laser Doppler flowmeter was placed on the left side of the dorsal surface of the spinal cord, avoiding superficial visible blood vessels. The optical output power of the He-Ne laser beam in the laser Doppler flowmeter (ALF 21, Advance Co.) was 2 mW at a wavelength of 780 nm. The flow signal was recorded with a 3 s time constant. After cessation of the experiment, the spinal level of the recording site was confirmed by exposing and tracing the spinal nerve roots.

Denervation of the baroreceptors. Bilateral carotid sinus nerves and cervical vagal nerves were surgically severed in 6 rats. Denervation of the baroreceptors was confirmed by disappearance of bradycardia in response to intravenous administration of phenylephrine. SCBF recording was started approximately 1 h after baroreceptor denervation.

Transsection of the spinal cord. The spinal cord was transected under anesthesia at the C1–2 level in 6 rats. SCBF recording was started approximately 1 h after spinal transection. The systolic blood pressure was kept above 80 mmHg by injection of 4% Ficoll 70 (Amersham Biosciences, Uppsala, Sweden) after spinal transection.

Administration of drugs. Alpha adrenoceptor blockade was induced in six rats by i.v. injection of 0.5 mg·kg⁻¹ phenoxybenzamine hydrochloride (Nacalai Tesque, Kyoto, Japan). Stimulation of alpha adrenoceptors was induced in six rats by i.v. injection of 3–20 µg·kg⁻¹ phenylephrine (Nacalai Tesque).

Statistical analysis. Data were expressed as mean ± SEM. Comparisons of group responses were made by analysis of variance (ANOVA), Dunnett’s multiple comparison test, and paired t-test. Probability values of less than 5% were considered significant.

RESULTS

SCBF responses to pinching of the hindpaw or forepaw

SCBF responses to pinching were investigated in 6 CNS-intact rats with basal SCBF and mean arterial pressure (MAP) of 26 ± 2 mV and 86 ± 6 mmHg, respectively. As shown in the sample recordings from one rat (Fig. 1), ipsilateral or contralateral pinching of either a hindpaw or forepaw produced increases in L4 dorsal SCBF. The augmentation of SCBF was greatest in response to pinching of the ipsilateral hindpaw as compared with responses from either forepaw and the contralateral hindpaw (Fig. 1, A, B, E, F) or a forepaw (C, D, G, H) ipsilateral (A–D) or contralateral (E–H) to the SCBF recording site. Horizontal bars indicated stimulus period of 30 s.

Figure 2 summarizes the responses of ipsilateral L4–6 dorsal SCBF (n = 6) to pinching of the hindpaw or the forepaw. The SCBF started to increase immedi-
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Fig. 1. Responses of ipsilateral L4–6 spinal cord blood flow (SCBF) and mean arterial pressure (MAP) to pinching of the ipsilateral hindpaw or forepaw. Pooled data (n = 6) were expressed as percentages of the value at Time = 0, the onset of stimulation. Closed circles represent means, vertical bars indicate SEM. * indicates p < 0.05 and ** indicates p < 0.01 per Dunnett test for significant difference from value at Time = 0.

Fig. 3. Responses of L4–6 spinal cord blood flow (SCBF) and mean arterial pressure (MAP) to pinching of the hindpaw or the forepaw. Response magnitudes at 10 s after the onset of pinching were compared. Pinching was applied to a hindpaw or a forepaw ipsilateral (shaded columns) or contralateral (white columns) to the SCBF recording site. ** indicates p < 0.01 per Dunnett test for significant difference from value at the onset of pinching. ## indicates p < 0.01 per paired t-test for significant difference between responses to ipsilateral and contralateral pinching or between responses to pinching of the hindpaw and the forepaw. n = 6.

SCBF responses after denervation of the arterial baroreceptors

The contribution of baroreceptor reflexes to the SCBF responses to pinching of the hindpaw and forepaw were examined in animals whose baroreceptors were denervated. In 6 rats after denervation of the baroreceptors,
basal SCBF and MAP were 30 ± 4 mV and 86 ± 4 mmHg, respectively. As shown in Fig. 4, increases of both SCBF and MAP evoked by pinching of the ipsilateral hindpaw or forepaw in baroreceptor-denervated animals were not significantly different from those of baroreceptor-intact animals.

SCBF responses to intravenous phenylephrine
The effects of arterial pressure increases on SCBF were investigated with phenylephrine, an alpha adrenoceptor stimulant. Doses of phenylephrine were adjusted in order to obtain MAP responses similar to those achieved with pinching.

In 6 rats treated with phenylephrine, basal SCBF and MAP were 26 ± 3 mV and 91 ± 7 mmHg, respectively. As shown in the sample recording (Fig. 5, A and B), intravenous phenylephrine (12 μg·kg⁻¹) increased SCBF and MAP. As summarized in Fig. 5, C and D, 3–20 μg·kg⁻¹ of phenylephrine increased MAP to 133 ± 3%, and SCBF to 112 ± 1% of pretreatment values, leading to significant decreases of SCBF/MAP to 84 ± 3% of pretreatment values.

SCBF responses after treatment with phenoxybenzamine
In order to eliminate systemic blood pressure responses to pinching, animals were treated with phenoxybenzamine, an alpha-receptor blocking agent. In 6 rats treated with phenoxybenzamine, pre-stimulation SCBF and MAP were 24 ± 2 mV and 57 ± 4 mmHg, respectively. Four of these 6 animals were among the control cohort for which results are summarized in Fig. 2. Hence, their responses to pinching were recorded before and after administration of phenoxybenzamine.

After treatment with phenoxybenzamine, responses of MAP to pinching were attenuated (Fig. 6, B, D, F and H), and the SCBF responses to pinching of the ipsilateral forepaw were abolished (Fig. 6, C and G). On the other hand, the SCBF responses to pinching of the ipsilateral hindpaw persisted (Fig. 6, A and E). The SCBF started to increase immediately after the onset of hindpaw pinching, and reached a maximum level (118 ± 4% of control values) at 20 s after the onset of stimulation. The SCBF returned to prestimulus levels within 20 s of the cessation of stimulation (Fig. 6E).

As summarized in Fig. 7, treatment with phenoxybenzamine abolished the responses of SCBF to pinching of the ipsi- or contra-lateral forepaw, and the contralateral hindpaw, and significantly attenuated responses in MAP.
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On the other hand, the increase in SCBF induced by pinching of the ipsilateral hindpaw persisted, although the magnitude was reduced. The ratio of SCBF/MAP in response to pinching of the ipsilateral hindpaw showed a significant increase (113 ± 4% of control values) after treatment with phenoxybenzamine.

**SCBF responses after spinalization**

The SCBF responses to pinching of the hindpaw and forepaw were investigated in rats whose spinal cords were severed at the C1–2 level. In these 6 rats, basal SCBF and MAP after spinalization were 32 ± 3 mV and 60 ± 3 mmHg, respectively.

As shown in the sample recordings in Fig. 8, after spinalization, MAP responses to pinching of the forepaw or the hindpaw were negligible (Fig. 8, B, D, F and H), and SCBF responses were observed only when pinching was applied to the ipsilateral hindpaw (Fig. 8A).

Figure 9 summarizes the responses of ipsilateral SCBF and MAP to pinching of the hindpaw or the forepaw in 6 spinalized rats. Pinching of the ipsilateral hindpaw increased SCBF to 132 ± 5% of prestimulus levels whereas pinching of the ipsilateral forepaw produced no significant responses. Pinching of the contralateral hindpaw or forepaw produced no significant responses in SCBF (Fig. 10A), with small increases in MAP (Fig. 10B). The SCBF/MAP showed significant increases (127 ± 6%) in
DISCUSSION

This study determined responses of SCBF to natural noxious stimulation of the skin (pinching). The stimulation was delivered to the same (hindpaw) or to distant (forepaw) segments, either ipsilateral or contralateral to the recording site of SCBF. Previous studies have examined the effects of noxious electrical stimulation of the somatic nerves, such as sciatic or femoral nerves [9–14], but none have compared the effects of segmental and extra-segmental stimulation on SCBF. The present study demonstrated that pinching of either the forepaw (extra-segmental stimulation) or the hindpaw (segmental stimulation) resulted in similar increases in MAP, but with quantitatively different effects on L4–6 dorsal SCBF. Specifically, hindpaw pinching resulted in larger increases in ipsilateral SCBF (Figs. 1, 2 and 3).

The increases in SCBF elicited by pinching of the ipsilateral hindpaw remained after spinalization and after alpha adrenergic blockade with phenoxybenzamine, despite minimal effects of pinching on MAP. This demonstrates that the input from the ipsilateral hindpaw exerts a local influence on SCBF. Specifically, since afferent input from the hindpaw enters the spinal cord at the L4–6 level in rats, it appears likely that the increases of ipsilateral L4–6 SCBF in response to hindpaw stimulation are segmentally organized responses. However, in this case these responses are unlikely to involve a significant contribution by sympathetic efferent nerves. Previous studies have demonstrated potent segmental and ipsilateral organization of the SCBF response to brushing [3, 4]. The increases in SCBF evoked by brushing were also independent of arterial blood pressure responses and supraspinal structures. Given that electrical stimulation of the femoral and/or the sciatic nerves has been shown to produce ipsilateral, segmental increases in spinal cord glucose metabolism [15, 16], collectively, the present and
previous studies suggest that mechanical cutaneous stimulation results in an ipsilateral, segmentally organized augmentation in SCBF, due largely to neuronal activation in the dorsal spinal cord. In addition, it has been reported that the increases of SCBF elicited by electrical stimulation of the somatic nerves cannot be explained solely by metabolic effects [13]. Possible contributions of local vasodilator neurons (including those releasing NO, CGRP and VIP) to these segmental increases of SCBF remain to be investigated.

The SCBF responses to pinching of the ipsilateral hindpaw in spinalized animals showed larger increases (132 ± 5% of prestimulus levels) than those after phenoxybenzamine treatment (118 ± 4% of prestimulus levels). This is congruent with a previous report that SCBF responses to brushing diminished after treatment with phenoxybenzamine. Furthermore, it was also shown that treatment with phenoxybenzamine had no effect on SCBF responses to brushing in spinalized animals [4]. These results suggest an alpha-receptor mediated augmentation of SCBF responses to ipsilateral and segmental stimulation via supraspinal structures.

Since pinching produced increases in systemic blood pressure, this study examined the contribution of baroreceptor reflexes to the increases of SCBF using baroreceptor-denervated rats, and showed that the increases of SCBF were not influenced via baroreceptor reflexes. These results are consistent with those reported by Marcus et al. [9] which showed that stimulation of baroreceptors via increased pressure in the carotid bifurcation had no influence on SCBF.

Brushing stimulation only increases ipsilateral segmental SCBF [3, 4]. On the other hand, the present study shows that SCBF increases in response to noxious mechanical stimulation of pinching applied not only to the ipsilateral hindpaw (segmental stimulation), but also to the extra-segmental forepaw or to the contralateral hindpaw. The responses of both SCBF and MAP to pinching of the forepaw or contralateral hindpaw were abolished by spinalization, demonstrating that these responses were mediated supraspinally. In this regard, it has previously been reported that the responses of MAP to pinching of the fore- or hindpaw are mediated supraspinally [7].

Pretreatment with phenoxybenzamine, an alpha-adrenergic blocker, significantly attenuated MAP responses to pinching and abolished SCBF responses to pinching of either the forepaw or contralateral hindpaw. These results suggest that the increases in SCBF following pinching of the forepaws or the contralateral hindpaw were secondary to elevation of MAP. However, the results of this study do not exclude the possibility that phenoxybenzamine directly blocked alpha-receptors in the spinal blood vessels.

MAP-mediated SCBF increases were examined with use of phenylephrine which does not pass through the blood-brain barrier [17]. After intravenous administration of phenylephrine at doses which produced increases of MAP similar to those induced by pinching, SCBF showed significant increases. The increases of SCBF induced by phenylephrine were smaller than those elicited by ipsilateral hindpaw pinching, however, they were comparable to the responses of SCBF to pinching of the forepaw (ipsi- or contra-lateral) or the contralateral hindpaw. These results also suggest that the increases of SCBF following pinching of the forepaw or contralateral hindpaw are secondary to augmentation of MAP.

Marcus et al. [9] reported that 40–50 mmHg increases in arterial pressure induced with phenylephrine did not change SCBF in anesthetized dogs, and attributed this to autoregulation. On the other hand, the present study showed small but significant increases of SCBF in response to blood pressure increases with phenylephrine. The discrepancy between our results and those by Marcus et al. is probably due to the methods of blood flow recording; the present study employed laser Doppler flowmetry which is extremely sensitive and able to detect even small blood flow changes in a variety of tissues, whereas Marcus et al. employed the microsphere method which cannot detect transient blood flow changes. Also, in the present study the ratio of SCBF/MAP, an indicator of vascular conductance, showed decreases in response to phenylephrine administration and pinching of the forepaw or contralateral hindpaw, suggesting a degree of vasoconstriction of the spinal vessels in response to these stimulations. This phenomenon may be one component of autoregulation. Korbine et al. [18] reported that autoregulation of SCBF is mediated via alpha-adrenoceptors and in the present study phenoxybenzamine abolished the decreases in SCBF/MAP.

Spinal alpha-adrenoceptors may be stimulated by the release of noradrenaline from the endings of the sympathetic nerves which innervate spinal blood vessels [19, 20]. It has been reported that hypothermia or impact injury, but not hypotension, changes SCBF via the peripheral sympathetic nerves [21–23]. Thus, it would appear that pinching of the forepaw or contralateral hindpaw elicits a reflex vasoconstriction (possibly via the sympathetic nerves) which would otherwise decrease SCBF, but that this reflex is overwhelmed by marked increases in MAP.

The ratio of SCBF/MAP showed no significant response to pinching of the ipsilateral hindpaw, while it increased significantly after treatment with phenoxybenzamine. These results suggest that the combined response of both vasoconstriction, presumably mediated by alpha adrenoceptors, and vasodilatation mediated by ipsilateral, segmental influences result in no significant net change in the ratio of SCBF/MAP.

In conclusion, the present study has demonstrated that noxious mechanical stimulation of the skin increases SCBF via at least two classes of mechanisms: general-
ized mechanisms which affect SCBF broadly and specific localized mechanisms. The generalized mechanisms include (1) systemic arterial pressure–dependent mechanisms, and (2) somato-sympathetic reflexes or autoregulation, both of which may be mediated via alpha-receptors. The specific localized mechanisms seem to involve metabolic effects induced by neuronal activation in response to ipsilateral, segmental inputs. This occurs against a backdrop of spinal vascular tone which may be reflexly up-regulated (or may be elicited via autoregulatory mechanisms due to elevation of systemic arterial pressure) by noxious mechanical cutaneous stimulation.

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