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SKIN BLOOD FLOW DISTURBANCES IN THE CONTRALATERAL LIMB IN A PERIPHERAL MONONEUROPATHY IN THE RAT

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Abstract—Electrical excitation of nociceptive afferents in an extremity has been demonstrated to increase skin blood flow in the contralateral extremity. Hence, one would expect that loose sciatic nerve ligation, which induces an experimental painful peripheral neuropathy, may also provoke a vasodilator response in the contralateral hindpaw. On the non-ligated side, such a response may involve inhibited skin vasoconstrictor activity as well as neurogenically mediated active vasodilation. We studied skin blood flow changes in the rat hindpaw consequent to contralateral loose sciatic nerve ligation. After ligation, we also investigated whether blockade of afferent input from the ligated sciatic nerve to the spinal cord, by means of lidocaine, overrules the vasodilator response in the non-ligated paw. On the non-ligated side, we assessed the vasoconstrictor response of skin microvessels to cooling of the rat abdomen as a measure of skin vasoconstrictor activity in this paw. In order to investigate the involvement of sensory and/or non-sensory nerve fibers in the non-ligated sciatic nerve on skin blood flow abnormalities in the non-ligated paw, we studied the influence of blockade of these fibers through successive capsaicin and lidocaine application. We show that loose ligation of the sciatic nerve induces a vasodilator response in the contralateral hindpaw, which is completely abolished by blockade of afferent input from the ligated sciatic nerve. From day 1 after ligation, skin vasoconstrictor activity in the non-ligated paw was reduced, as indicated by an impaired vasoconstrictor response to cooling of the rat abdomen. Besides, blockade of sensory but not of non-sensory nerve fibers on the non-ligated side attenuated the vasodilator response in this paw.

The data presented here indicate that loose ligation of the rat sciatic nerve induces a vasodilator response in the contralateral hindpaw. On the non-ligated side, this vasodilator response may involve inhibition of skin vasoconstrictor activity, as well as antidromically acting sensory nerve fibers. Copyright © 1996 IBRO. Published by Elsevier Science Ltd.

Key words: chronic constriction injury, rats, reflex sympathetic dystrophy, skin blood flow, contralateral extremity, nerve block.

Blumberg and Wallin⁶ have demonstrated that, in humans, painful intraneural electrical stimulation increases skin blood flow (SBF) not only ipsilaterally, but also in the contralateral extremity. Since the vasodilator response in the non-stimulated extremity occurred only when stimulation strength was high enough to cause sharp and localized pain, the authors proposed that the afferent limb of this reflex involves excitation of A- δ fibers. Likewise, Loven³² demon-

strated that, in rabbits, electrical stimulation of cutaneous nerves induces dilation of cutaneous vessels in the contralateral extremity.

Loose ligation of a sciatic nerve in rats (chronic constriction injury, CCI) induces an experimental painful peripheral neuropathy that reproduces many of the signs and symptoms observed in clinical conditions of neuropathic origin, such as causalgia and reflex sympathetic dystrophy.^{4,5,38,44} Additionally, this nerve constriction injury has been demonstrated to cause excitation of nociceptive afferents.^{23,51} Hence, one would expect that loose sciatic nerve ligation may also induce a vasodilator response in the contralateral hindpaw. Such a phenomenon would

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Abbreviations: CCI, chronic constriction injury; CPT, cold pressor test; LDF, laser Doppler flowmetry; PU, perfusion units; SBF, skin blood flow.

render this paw unfit to serve as a control for studies on changes in SBF and skin temperature on the ligated side.^{4,46} Therefore, we measured SBF in the rat hindpaw before and after contralateral loose sciatic nerve ligation. To test the hypothesis that SBF abnormalities in the rat hindpaw induced by contralateral loose sciatic nerve ligation indeed result from increased afferent input from the loosely ligated sciatic nerve to the spinal cord, we studied the influence of blockade of the loosely ligated sciatic nerve, by means of lidocaine, on SBF in the non-ligated hindpaw.

A vasodilator response on the non-ligated side may result from reduced (sympathetic) skin vasoconstrictor activity in this paw. It has been demonstrated previously that activation of nociceptive afferents may reduce skin vasoconstrictor activity.^{15,18,21} Therefore, we assessed, on the non-ligated side, as an indirect measure of skin vasoconstrictor activity, the effectiveness of the vasoconstrictor response of skin microvessels to cooling of the rat abdomen. It has been demonstrated by Linderöth *et al.*³¹ that this vasoconstrictor response is impaired in the case of reduced (sympathetic) skin vasoconstrictor activity.

On the non-ligated side, a neurogenically mediated vasodilator response may involve antidromically acting sensory (C and A- δ) and/or orthodromically acting sympathetic nerve fibers. This consideration prompted us to examine the influence of blockades of both types of nerve fibers in the non-ligated sciatic nerve on the vasodilator response in the non-ligated hindpaw. Firstly, the capability of sensory (C-nociceptor and A- δ) nerve fibers to mediate a neurogenic inflammatory (vasodilator) response was blocked by means of perineural application of capsaicin.^{8,10,19,27} Thus, if capsaicin would attenuate a vasodilator response induced by contralateral loose sciatic nerve ligation, it is likely that sensory nerve fibers in the non-ligated sciatic nerve are involved in this response. Following capsaicin application, impulse propagation of non-sensory nerve fibers was blocked by means of lidocaine to explore the involvement of these fibers in the vasodilator response induced by contralateral loose sciatic nerve ligation.

EXPERIMENTAL PROCEDURES

Fifty-eight male Lewis rats obtained from our own animal facilities, weighing 250–350 g, were used at the start of the experimental protocol. This protocol was approved by the Institutional Animal Care Committee of the University of Limburg, The Netherlands.

Surgery

The operative procedure has been described previously by Bennett and Xie.⁵ Briefly, the animals were anesthetized with 100 mg/kg ketamine hydrochloride intraperitoneally (Nimatek, AUV, Cuijk, The Netherlands) and 0.5 mg diazepam subcutaneously (Centrafarm BV, Etten-Leur, The Netherlands). Administration of ketamine hydrochloride was repeated (30 mg/kg, i.p.) if necessary. The operative procedure was performed under aseptic conditions. The right sciatic nerve was exposed from high-thigh to mid-thigh

level, just proximal to the trifurcation into the tibial, sural and peroneal nerves. Using a dissecting microscope ($\times 25$ magnification), the sciatic nerve was loosely ligated with four chromic cat gut ligatures (4-0, Ethicon, Norderstedt, Germany), just proximal to the trifurcation, with a spacing of about 1 mm. The wound was closed with mersilene muscle sutures (5-0, Ethicon) and mersilene skin sutures (2-0, Ethicon).

Laser doppler flowmetry

SBF was assessed by means of laser Doppler flowmetry (LDF), because it can detect instantaneous changes³¹ and the flow signal obtained by this technique has been demonstrated to be influenced by sympathetic vasoconstrictor⁵⁰ as well as antidromic vasodilator mechanisms.^{12,33} The technique has been described in detail elsewhere.³⁷ Briefly, LDF evaluates the Doppler shift of laser light backscattered by moving blood cells and provides a measure of flow. In human skin, the penetration depth of the laser light contributing to the signal is about 0.6 mm.³⁶ In a pilot study in four rats, we measured the thickness of the skin in a biopsy which was obtained at the standard site of blood flow measurement (see below). Two biopsies were obtained three weeks after ipsilateral loose sciatic nerve ligation, whereas the two remaining biopsies were obtained from age-matched control animals. Skin thickness always exceeded 0.9 mm. Hence, in our experimental set-up blood cell movements were most likely recorded solely from the microvasculature of the skin. Measurements were executed with a commercially available system (Perimed PF3 with standard angled probe (90°), Perimed, Linköping, Sweden). The sample volume was about 1 mm³. The following settings were used: time constant of 2 s, to avoid heart beat oscillations in the signals; low band pass filter 'on', in order to reduce movement artifacts; frequency limited to the 0.07–12 kHz band. An output of 1 V was calibrated against 100 perfusion units (PU).

Experimental protocol skin blood flow measurements

For each measurement, the animals were anesthetized as described above with ketamine hydrochloride (100 mg/kg, i.p.) and diazepam (0.5 mg, s.c.). In a few cases, injections of ketamine hydrochloride were supplemented (30 mg/kg, i.p.). Throughout the measurement body temperature was kept at 37°C, using an infrared heating lamp controlled by a thermoanalysis system (Hugo-Sachs Elektronik, March-Hugsteden, Germany). The ambient temperature was maintained at 21–23°C. To measure SBF on the non-ligated side, each animal was placed in the prone position on a plastic floor, which was adjustable in height. The plantar surface of this hindpaw was immobilized with adhesive tape. The abdomen of the rat was placed over an opening in the plastic floor with a diameter of 5 cm, through which cooling could be applied over a standardized area of abdominal skin for the cold pressor test (CPT).

Subsequently, LDF was employed to assess SBF. Measurements were performed at a prominence located on the lateral side of the plantar surface of the rat hindpaw. Within this specific area, the probe was moved about in order to determine maximal basal SBF. The skin area under investigation is innervated by the tibial nerve, which originates from the sciatic nerve.¹⁴ A micromanipulator (Leitz 115001, Leica BV, Rijswijk, The Netherlands) was used to position the standard angled probe closed to the surface of the site of measurement.

In a first group of 20 rats (group I), LDF measurements were performed one day before, as well as one, three, five, seven, 14, 21 and 28 days after ligation of the contralateral sciatic nerve. This protocol was chosen in order to assess the time-course of changes in SBF induced by contralateral loose sciatic nerve ligation, if any. At these time-points, SBF was measured for a period of 3 min after stabilization of the signal for at least 3 min. Subsequently, the abdomen of the

rat was cooled by spraying ethyl chloride (Medica BV, Hertogenbosch, The Netherlands) for 5 s (CPT). After the CPT, SBF was monitored for an additional 3 min. In all experiments, SBF had reached its lowest value within these 3 min. The ratio of mean SBF before the CPT divided by the lowest value after the CPT was calculated to evaluate the effectiveness of the skin vasoconstrictor response. A ratio lower than that found in the pre-ligation situation is indicative of reduced skin vasoconstrictor activity.³¹

In a second group of six unoperated rats (group II), LDF was employed to obtain information on the effects of repeated measurements and increased age/weight on SBF. To this end, these rats were subjected to the same experimental protocol as the rats in group I.

In a third group of eight rats (group III), we measured SBF on the non-ligated side one day before contralateral loose sciatic nerve ligation, as well as five days after this procedure. At day 5 after ligation, SBF was measured on the non-ligated side before and after exposure of the (contralateral) loosely ligated sciatic nerve. Subsequently, 2% lidocaine (AUV, Cuijk, The Netherlands) was applied to the loosely ligated sciatic nerve in such a way that part of this nerve proximal to the ligatures was continuously (over a 1-h period) covered with a layer of this solution. In previous studies in the CCI model, a 1% lidocaine solution has been applied to the loosely ligated sciatic nerve in order to block afferent input from this nerve to the spinal cord.^{9,16} We opted for a 2% lidocaine concentration to ensure total blockade of afferent input. The total amount of lidocaine used never exceeded 1 ml. SBF was determined again 10 and 60 min later.

In a fourth group of eight rats (group IV), SBF was measured on the non-ligated side one day before contralateral loose sciatic nerve ligation, as well as five days after this procedure. At day 5 after ligation, SBF measurements were performed on the non-ligated side before and after exposure of the (ipsilateral) non-ligated sciatic nerve. Following exposure of the non-ligated sciatic nerve, the capability of sensory fibers in this nerve to mediate a neurogenic inflammatory (vasodilator) response was abolished. To this end, capsaicin (Sigma Chemical Company, St Louis, MO, U.S.A.) was applied perineurally at mid-thigh level.¹² To prevent systemic effects of capsaicin, a thin layer of plastic foil was placed underneath a segment of the exposed sciatic nerve, after which a piece of cotton containing the 1% capsaicin solution was applied to this nerve. Capsaicin was used because it has been demonstrated repeatedly to provoke a profound and apparently irreversible abolition of the capability of sensory fibers to mediate a neurogenic inflammatory response.^{8,10,19,27} In several electrophysiological studies, it has been shown that the neurotoxic effects of capsaicin on impulse propagation are specific to primary afferent nerve fibers.^{2,17,20,35,43} It has also been demonstrated that capsaicin does not exert effects on non-sensory (i.e. autonomic and motor) nerve fibers.^{7,11,19} The cotton containing the capsaicin solution was removed after 15 min, after which excess capsaicin solution was absorbed with a dry piece of cotton.^{29,48} SBF was assessed again 10 and 60 min after the 15-min capsaicin application.

Following this 1-h observation period (during which the influence of capsaicin application on SBF was assessed), we applied a 2% lidocaine solution to the capsaicin-pretreated non-ligated sciatic nerve to block impulse propagation of non-sensory nerve fibers. Lidocaine has been demonstrated previously to block not only sensory, but also non-sensory (motor and sympathetic) nerve fibers.^{13,24,30,45} It has also been demonstrated in isolated rat sciatic nerve that perineural capsaicin application potentiates the effect of subsequent lidocaine application.⁴¹ Since no evidence has so far been presented for the involvement of fibers other than sensory and sympathetic nerve fibers in neurogenic skin vasomotor control, the effect of lidocaine application to capsaicin-pretreated nerves on SBF abnormalities can most

likely be attributed to blockade of impulse propagation of sympathetic nerve fibers. Again, lidocaine was applied to the nerve in such a way that part of the nerve was continuously (over a 60-min period) covered with a layer of this solution. The total amount of lidocaine used never exceeded 1 ml. SBF was again determined 10 and 60 min later.

In a fifth group of eight rats (group V), LDF was employed to obtain information on systemic effects of lidocaine application on SBF abnormalities induced by contralateral loose sciatic nerve ligation. To this end, SBF was assessed in the non-ligated hindpaw before ligation and at day 5 after ligation. At day 5, SBF was assessed before and after creation of a surgical wound in the left forepaw of the rats. To this end, the triceps brachii muscle was dissected bluntly. The size of this surgical wound in the forepaw was comparable to the size of the surgical wound in the hindpaw used for the application of capsaicin and lidocaine to the sciatic nerve. Lidocaine was then applied in such a way that a layer of the solution was continuously present in this wound over a 60-min period. The total amount of lidocaine used was 1 ml for each experiment. SBF measurements in the non-ligated hindpaw were repeated 10 and 60 min later.

In another group of eight rats (group VI), LDF was employed to obtain information on the effects on SBF of capsaicin and lidocaine application to non-ligated sciatic nerves. To this end, capsaicin and lidocaine were applied consecutively, as described above. SBF was measured before and after exposure of the sciatic nerve. Following exposure, SBF was measured at 10 and 60 min following the 15-min capsaicin application, and at 10 and 60 min after the start of subsequent lidocaine application.

Statistics

The LDF data are expressed relative to their control values, i.e. the value of interest divided by the corresponding pre-ligation values. These data are presented as medians with their interquartile ranges, since the data obtained were not normally distributed. Non-parametric analysis of variance was performed for resting SBF and the vasoconstrictor response to the CPT using the Friedman test. At first this was done for all time-points together and subsequently for subgroups. In cases where two time-points were compared, we used the Wilcoxon signed ranks test for paired data to test for significant differences. Differences were regarded statistically significant when $P < 0.05$.

RESULTS

Figure 1 shows that, in group I, when compared with pre-ligation values (normalized value = 1, interquartile range of absolute SBF values, 50–120 PU), loose ligation of the sciatic nerve induced an increase in SBF on the non-ligated side from day 3 after ligation onwards ($P < 0.001$), stabilizing at a plateau after day 7.

As illustrated in Fig. 2, when compared with pre-ligation values (normalized value = 1, interquartile range of absolute values, 1.49–2.38) loose ligation of the right sciatic nerve induced ($P < 0.01$) an impairment of the vasoconstrictor response to the CPT from day 3 onwards, with the exception of day 14.

Table 1 shows that, in group II (unoperated rats), when compared with the initial values (normalized value = 1, interquartile range of absolute SBF values, 41–107 PU), during the whole observation period no significant changes in mean SBF were observed. The data in Table 1 additionally demonstrate that, when

SKIN BLOOD FLOW NON-LIGATED SIDE changes in time

Fig. 1. LDF values as measured in the non-ligated hindpaw before loose ligation of the sciatic nerve in the other hindpaw, as well as at several time-points after this procedure. All data are normalized to pre-ligation values (= 1), and presented as medians (dots) and interquartile ranges (error bars). Ordinate: SBF. Abscissa: the different time-points. XXX $P < 0.001$, as compared to pre-ligation SBF values.

VASOCONSTRICTOR RESPONSE TO CPT changes in time

Fig. 2. Vasoconstrictor response to CPT of skin microvessels as measured in the non-ligated hindpaw before loose ligation of the sciatic nerve in the other hindpaw, as well as at several time-points after this procedure. All data are normalized to pre-ligation values (= 1), and presented as medians (dots) and interquartile ranges (error bars). Ordinate: the vasoconstrictor response. Abscissa: the different time-points. XX $P < 0.01$, as compared to pre-ligation values.

compared with the initial values (normalized value = 1, interquartile range of absolute values, 1.38–2.12), during the whole observation period no significant changes were observed in the vasoconstrictor response to the CPT.

Figure 3 demonstrates that, in group III, when compared with pre-ligation values (normalized value = 1, interquartile range of absolute SBF values, 48–116 PU), SBF on the non-ligated side was increased at day 5 (3.51, $P < 0.01$). Exposure of the

loosely ligated sciatic nerve did not change contralateral SBF (3.04). Subsequent blockade of the afferent input from the loosely ligated sciatic nerve to the spinal cord by means of lidocaine resulted in a decrease in SBF on the non-ligated side. As compared to post-exposure values, SBF was reduced both at 10 (1.44, $P < 0.01$) and 60 min (1.32, $P < 0.01$) after the start of the lidocaine application. Moreover, at both time intervals, SBF values no longer differed from pre-ligation values.

Table 1. Time-course (days) of mean skin blood flow (in perfusion units) and vasoconstrictor response to the cold pressor test; normalized median values and interquartile ranges are presented

	Initial values	Day					28
		1	3	5	7	14	
Mean SBF	1	1.15 (0.68–1.51)	1.08 (0.65–1.44)	0.92 (0.63–1.22)	0.98 (0.77–1.18)	0.92 (0.78–1.27)	1.12 (0.8–1.41)
Vasoconstrictor response	1	1.11 (0.62–1.61)	0.83 (0.67–1.21)	0.97 (0.77–1.18)	1.21 (0.88–1.46)	0.79 (0.54–0.87)	1.2 (0.71–1.48)

Figure 4 shows that, in group IV, when compared with pre-ligation values (normalized value = 1, interquartile range of absolute SBF values, 27–75 PU), SBF on the non-ligated side was increased at day 5 (3.32; $P < 0.05$). Exposure of the non-ligated sciatic nerve did not change ipsilateral SBF (3.83). Subsequent application of capsaicin to the non-ligated sciatic nerve resulted in a temporary increase in ipsilateral SBF immediately upon application (data not shown). This increase, however, reached a maximum within several minutes after the removal of the piece of cotton containing the capsaicin solution. Thereafter, the initial increase was followed by a decrease in ipsilateral SBF. As a result, when compared with post-exposure values, SBF was decreased following capsaicin application, being 2.81 at 10 min ($P < 0.05$) and 2.77 at 60 min ($P < 0.05$). Never the less, SBF values remained increased at 10 ($P < 0.05$) and 60 min ($P < 0.05$) when compared with pre-ligation values. Figure 4 also demonstrates that subsequent application of lidocaine did not influence SBF, being 2.38 at 10 min and 2.50 at 60 min. As a result, SBF values remained increased at 10 min ($P < 0.05$), when compared with pre-ligation values.

In group V, when compared with pre-ligation values (normalized value = 1, interquartile range of absolute SBF values, 25–79 PU), SBF on the non-ligated side was increased at day 5 (3.54; $P < 0.05$). Creation of a surgical wound in the forepaw did not influence SBF values (2.85) in the non-ligated hindpaw. As compared to the values obtained after creation of this surgical wound, subsequent application of lidocaine into the wound did not change SBF in the non-ligated hindpaw, being 3.02 at 10 min and 1.89 at 60 min after the lidocaine application.

In group VI (non-ligated rats), SBF before exposure of the sciatic nerve (normalized value = 1, interquartile range of absolute SBF values, 26–48 PU) did not differ from post-exposure values (0.86). As was the case in group IV, subsequent application of capsaicin to the sciatic nerve resulted in a temporary increase in ipsilateral SBF immediately upon application (data not shown). This increase again reached a maximum within several minutes after the removal of the piece of cotton containing the capsaicin solution. Thereafter, the initial increase in SBF subsided, as a result of which SBF did not differ from post-exposure values, being 1.0 at 10 min and 1.23 at 60 min after capsaicin application. After subsequent lidocaine application, SBF again did not differ from post-exposure values, being 0.95 at 10 min and 1.09 at 60 min after lidocaine application.

DISCUSSION

This study shows that loose ligation of the rat sciatic nerve induces an increase in SBF in the contralateral hindpaw from day 3 after ligation onwards. Blockade of afferent input from the loosely ligated sciatic nerve to the spinal cord completely

SKIN BLOOD FLOW NON-LIGATED SIDE blockade afferent input ligated side

Fig. 3. LDF values as measured in the non-ligated hindpaw before ligation and at day 5. At this time-point before and after exposure of the loosely ligated sciatic nerve and at 10 and 60 min after blockade of afferent input from the ligated sciatic nerve to the spinal cord (designated lidocaine). All data are normalized to pre-ligation values (= 1), and presented as medians (dots) and interquartile ranges (error bars). Ordinate: SBF. Abscissa: the different time-points. XX $P < 0.01$, as compared to values obtained before ligation; ++ $P < 0.01$, as compared to values obtained after exposure of the non-ligated sciatic nerve.

SKIN BLOOD FLOW NON-LIGATED SIDE capsaicin/lidocaine to non-ligated nerve

Fig. 4. LDF values as measured in rat skin before ligation and at day 5. At this time-point before and after exposure of the sciatic nerve, at 10 and 60 min after blockade of C-nociceptor nerve fibers (designated capsaicin), and at 10 and 60 min after blockade of conduction of other than C-nociceptor nerve fibers (designated lidocaine). All data are normalized to pre-ligation values (= 1), and presented as medians (dots) and interquartile ranges (error bars). Ordinate: SBF. Abscissa: the different time-points. X $P < 0.05$, as compared to values obtained before ligation; + $P < 0.05$, as compared to values obtained after exposure of the non-ligated sciatic nerve.

interrupts this vasodilator response. On the non-ligated side, the vasodilator response may involve inhibited skin vasoconstrictor activity, as well as release of vasodilator neuropeptides at the periphery of antidromically acting sensory nerve fibers.

Blockade of conduction of the loosely ligated sciatic nerve just proximal to the ligatures completely

interrupted the vasodilator response on the non-ligated side. This indicates that this response results from an increased level of afferent input from the loosely ligated sciatic nerve to the spinal cord. This finding agrees with the results of Blumberg and Wallin,⁶ who also demonstrated that local anesthesia of a nerve proximal to its stimulation site completely

interrupted the contralateral vasodilator response. Our findings also indicate that not only electrical,^{6,32} but also other forms of excitation of nociceptive afferents (such as loose sciatic nerve ligation) may induce a vasodilator response in the contralateral extremity.

In the non-ligated hindpaw of rats subjected to contralateral loose sciatic nerve ligation, we observed an impaired vasoconstrictor response of skin microvessels to cooling of the rat abdomen. This observation is indicative of reduced skin vasoconstrictor activity,³¹ which may contribute to the observed increase in SBF on the non-ligated side. The latter hypothesis is supported by our observation that SBF on the non-ligated side remained increased even after blockade (by means of lidocaine) of the non-ligated sciatic nerve. It has been demonstrated previously that noxious stimulation of nociceptors may cause inhibition of skin vasoconstrictor activity.^{15,18,21} The present study, however, is the first to demonstrate that activation of nociceptive afferents causes inhibition of skin vasoconstrictor activity in the contralateral extremity. This crossed spinal response may be related to the observation that loose ligation of a sciatic nerve induces changes in spinal cord neural activity not only in the ipsilateral,^{3,47} but also in the contralateral, dorsal horn.³⁴

Capsaicin application to the non-ligated sciatic nerve attenuated the vasodilator response induced by contralateral loose sciatic nerve ligation. This finding suggests that antidromically acting sensory nerve fibers in the non-ligated sciatic nerve are involved in this vasodilator response. Such a neurogenically mediated vasodilator response may involve antidromically acting unmyelinated (C) as well as small myelinated (A- δ) sensory nerve fibers.²² Subsequent application of lidocaine to the non-ligated sciatic nerve, in which the capability of sensory nerve fibers to mediate a neurogenic inflammatory (vasodilator) response had been blocked by capsaicin, did not influence the vasodilator response induced by contralateral loose sciatic nerve ligation. Hence, on the non-ligated side this response does not seem to involve fibers other than sensory nerve fibers.

As alluded to before, excitation of primary afferent sensory neurons may provoke release of vasodilator neuropeptides from their peripheral terminals.^{12,22,28,40} Hence, a third mechanism which may account for the observed increase in SBF on the non-ligated side could be circulation of these neuropeptides from the ligated to the non-ligated extremity. In our study, we observed that interruption of afferent input from the injured sciatic nerve to the spinal cord by means of lidocaine application completely interrupted the contralateral vasodilator response. This finding seemingly argues in favor of neural mediation across the spinal cord. Never the less, one should realize that lidocaine application to the injured sciatic nerve may simultaneously block release of vasodilator neuropeptides from its

peripheral terminals. Blockade of sensory nerve fibers in the non-ligated sciatic nerve (through application of capsaicin) attenuated the vasodilator response on the non-ligated side, which suggests that this response is at least partly neurally mediated. However, given the observation that SBF on the non-ligated side remained increased even after complete blockade of the non-ligated sciatic nerve (through application of lidocaine), we cannot exclude that a humoral component is also involved in the development of the contralateral vasodilator response. In line with the findings of our study, Levine *et al.*²⁹ demonstrated that excitation of primary afferent sensory neurons, following unilateral hindlimb injury, provokes an acute inflammatory response in the contralateral hindpaw. Interruption of venous circulation of the injured limb did not alter the contralateral response, whereas acute denervation of either limb significantly attenuated the contralateral response. These findings argue against the hypothesis of humoral mediation of contralateral inflammatory responses.

In the unoperated rats, no significant changes were observed in mean SBF or in the vasoconstrictor response to the CPT during the whole observation period. These findings indicate that the SBF abnormalities in the ligated group indeed result from loose ligation of the contralateral sciatic nerve. They also indicate that, in our study, repeated LDF measurements and increasing age/weight did not have any effect on SBF.

In both ligated and non-ligated rats, we observed a transient increase in ipsilateral SBF immediately upon application of capsaicin to the non-ligated sciatic nerves, which reached its maximum a few minutes after the removal of the piece of cotton containing the capsaicin solution. This transient ipsilateral increase, which has been described previously by Gamse and Saria,¹² is likely to represent the acute excitation phase during which an antidromic barrage is created, as a result of which vasodilator neuropeptides are released from the terminals of primary afferent nerve fibers.

Application of capsaicin as well as lidocaine to the sciatic nerve of non-ligated rats did not cause lasting changes in SBF readings. This finding suggests that in these anesthetized rats blood flow in the cutaneous vasculature is not neurogenically controlled. The latter hypothesis is in line with the findings of Willette *et al.*,⁵⁰ who demonstrated that the vasoconstrictor tone in the cutaneous vasculature of rats is predominantly mediated by a humoral action of circulating catecholamines at post-junctional α -adrenoceptors.

Loose ligation of a sciatic nerve in rats, which activates nociceptive afferents,^{23,51} induces an increase in SBF in the contralateral extremity. Other studies have demonstrated that loose ligation of a sciatic nerve induces sensory abnormalities in the contralateral hindpaw.^{1,25,42} In line with these findings, Levine *et al.*²⁹ demonstrated that a standard

injury stimulus (subcutaneous injection of normal saline into the foot pad), which also activates nociceptive afferents, induces sensory abnormalities and increased vascular permeability in the contralateral hindlimb of the rat. These contralateral responses, referred to as reflex neurogenic inflammation, also involved antidromically acting nociceptive afferents in the non-injured extremity.²⁹ The similarity between their findings and ours suggests that similar mechanisms are involved in these contralateral responses.

We applied chemical conduction blockades instead of surgical transection of nerves (sympathectomy and/or rhizotomy) for several reasons. Firstly, the use of chemical conduction blockades enabled us to assess SBF continuously, before and after application of the nerve conduction blockade, while the probe of the laser Doppler flowmeter remained in the same position. Thus, spatial variation is prevented and temporal variation can be kept to a minimum. Secondly, cutting of damaged nerves has been reported to provoke prolonged afterdischarges, both proximally and distally,^{39,49} which may influence SBF readings.

In line with our findings in this experimental animal model of neuropathic pain, we demonstrated

SBF abnormalities in the clinically non-affected extremities of reflex sympathetic dystrophy patients.²⁶ This congruity further attests to the usefulness of the CCI model in the evaluation of pathophysiological mechanisms underlying reflex sympathetic dystrophy.

CONCLUSION

The present study shows that loose ligation of the rat sciatic nerve induces an increase in SBF in the contralateral hindpaw from day 3 after ligation onwards. Blockade of afferent input from the loosely ligated sciatic nerve to the spinal cord interrupts this vasodilator response. On the non-ligated side, the vasodilator response may involve inhibited skin vasoconstrictor activity, as well as release of vasodilator neuropeptides at the periphery of antidromically acting sensory nerve fibers. The results of these experiments may have important implications for the practice of using one extremity as a control for studies on SBF, and probably also skin temperature, on the (contralateral) ligated side.

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