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RECOVERY OF NOCICEPTIVE FUNCTION IN THE DENERVATED SKIN: LONGITUDINAL STUDIES USING SCANNING LASER DOPPLER IMAGING AND VASCULAR LABELING

Summary of Ph.D. Thesis

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II. **Lakatos Sz**; Jancsó G; Horváth Á; Dobos I; Sántha P (2020) Longitudinal Study of Functional Reinnervation of the Denervated Skin by Collateral Sprouting of Peptidergic Nociceptive Nerves Utilizing Laser Doppler Imaging; Frontiers in Physiology (1664–042X): 11 Paper 439. 10. p., 2020,
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INTRODUCTION

The somatosensory system responds to a wide range of mechanical, chemical and thermal stimuli. This information is critical for the individual to be able to adapt to different conditions and challenges of the external and internal environment. Neuronal circuits consisting of a wide variety of neurons are responsible to receive and transmit this information from the periphery towards the central processing areas of the brain. Although these neuronal networks are vulnerable and any damage may lead to functional loss, they could demonstrate remarkable plasticity, may retain their responsiveness after prolonged deprivation of peripheral input and possess the ability to regenerate and reinnervate target tissues after injury. Significant efforts have been exerted to study the regenerative properties of peripheral sensory nerves and to find ways to facilitate functional regeneration. Our aim was to study the regenerative properties of the peripheral sensory nerve fibers innervating the cutaneous region of the rat hind paw following chemical and mechanical injuries. Restitution of cutaneous sensory functions may be achieved through distinct regenerative mechanisms. In our experiments we studied the collateral reinnervation through the invasion of intact axons from cutaneous areas adjacent to the denervated region. Intact chemosensitive primary afferent neurons besides their classical sensory afferent function, also possess sensory efferent function, a special feature characterized by neurogenic inflammatory response comprising of two main components, neurogenic plasma protein extravasation and sensory vasodilatation due to the release of neuropeptides, with a key role attributed to substance P (SP) and calcitonin gene-related peptide (CGRP) upon chemical or electrical stimulation. The presence of these neurogenic vascular reactions indicate the functional integrity of the sensory nerves innervating those cutaneous areas. As the cutaneous areas displaying antidromic stimulation-induced neurogenic vasodilation exactly coincide with the innervation territory of the stimulated nerve, the topographical localization of chemically induced vasodilation by laser Doppler imaging (LDI) technique is suitable to assess the pattern of cutaneous innervation and functional integrity of the corresponding nerves. However, these sensory efferent responses are completely abolished after denervating the skin, thus, measuring sensory neurogenic vasodilation is a reliable method also for the assessment of the extent and topography of the denervation and the subsequent functional regeneration of cutaneous sensory fibers. This approach allows a direct and objective evaluation of these biological processes without the need to interrogate complex nociceptive reflexes (animal studies) or apply subjective sensory testing (human studies). In this study the time course and spatial distribution

of chemically induced neurogenic vasodilatation was evaluated by visualizing changes in cutaneous blood flow as assessed by laser Doppler perfusion imaging following chemical or mechanical injuries affecting the peripheral sensory nerves innervating the rat hind paw. We aimed to evaluate and validate scanning laser Doppler imaging as a non-invasive method to longitudinally study the functional regeneration of cutaneous nociceptive nerves.

AIMS

The overall aim of this study was to examine the mechanisms of functional reinnervation of the denervated skin by using novel techniques utilizing scanning laser Doppler imaging and the vascular labeling method which enable the longitudinal observation of the reinnervation process. The specific objectives of this study were as follows:

1. To evaluate and validate a non-invasive method, scanning laser Doppler imaging for longitudinal study of the function of cutaneous peptidergic nociceptive afferent nerves.
2. To evaluate scanning laser Doppler imaging for the longitudinal study of the functional reinnervation, by nociceptive peptidergic afferent nerves, of the denervated skin.
3. To examine the functional reinnervation, by nociceptive peptidergic afferent nerves, of the denervated skin following perineural capsaicin treatment of peripheral nerves using scanning laser Doppler imaging and the vascular labeling technique.

MATERIALS AND METHODS

1. Animals and surgical interventions

All experiments were approved by the Ethics Committee for Animal Care at the University of Szeged as per the Council Regulation of 40/2013 (II. 14.) and were carried out in full accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering. The number of experimental animals was kept as low as possible.

Adult male Wistar rats (n = 34) weighing 250–280 g were used in the experiments. For all surgical interventions a combination of ketamine (Calypsol, 70 mg/kg, Gedeon Richter, Budapest, Hungary) and xylazine (CP-Xylazin 2%, 10 mg/kg, Produlab Pharma, Raamsdonksveer, Netherlands) was administered intraperitoneally to anaesthesize the animals. Animals were randomly assigned to go under either peripheral nerve transection or perineural capsaicin treatment according to two experimental designs. After each surgery and in between each LDI measurement the animals were re-housed.

Experimental design 1:

The animals underwent two surgeries 5 weeks apart. First, the right saphenous nerve was exposed, ligated and transected distal to the ligature, then a 0.5 cm long nerve segment of the distal stump was removed to prevent regenerative regrowth. The second surgery was performed on the same animals 40 days after transecting the saphenous nerve. This time the right sciatic nerve was exposed and treated perineurally by wrapping the nerve around with gelfoam soaked with 0.1 mL of a 1% solution of capsaicin (Sigma, Saint Louis, MO, USA; dissolved in saline containing 6% ethanol and 8% Tween 80).

Experimental design 2:

First, the right saphenous nerve was exposed and to chemically denervate the nerve, it was perineurally treated with 1% capsaicin solution. The second surgery was performed 7 weeks later, when the previously capsaicin treated saphenous nerve was transected and ligated.

2. Measurement of cutaneous blood perfusion with scanning laser Doppler flowmetry

Cutaneous blood perfusion of the dorsum of the rat hindpaw was measured by scanning laser Doppler flowmetry by capturing consecutive perfusion images with a PeriScan PIM3 scanning laser Doppler imager (Perimed, Järfälla, Sweden). For LDI scans the rats were anaesthetized with a combination of ketamine and xylazine administered as an intraperitoneal injection and then were placed on a heating pad to keep their body temperature relatively constant at 37±0.5°C while keeping the room temperature also constant at 22–23°C. The dorsal surfaces of both hindpaws were scanned by using the repeated scan mode with 52x42 pixel frame size, with the distance of the scanner aperture from the skin surface of 19 cm. The scanner was positioned to ensure that the laser beam was perpendicular to the skin surface.

The hindpaws were subjected to perfusion measurements every 2 minutes. Each of the total measurement sessions took 15–20 minutes for each animal. The baseline perfusion of unstimulated skin was recorded, then 5% mustard oil (in liquid paraffin) was applied on the unshaved hairy skin of the paws using a cotton swab. Immediately after the epicutaneous mustard oil application, the perfusion measurement was continued.

All flow values were expressed as means ± S.E.M. Basal tissue perfusion and changes in blood flow induced by mustard oil were recorded in arbitrary perfusion units (PU) and expressed as per cent change relative to baseline. Baseline values were obtained by calculating the average of three subsequent measurements before the application of mustard oil. For quantitative evaluation, images displaying the maximum vasodilatatory responses were used in each experiment.

Scanning laser Doppler images were taken before surgery and repeatedly during the follow-up periods after each surgeries.

3. Quantitative evaluation of the cutaneous denervation and reinnervation by using LDI perfusion images captured from stimulated skin areas

The innervation territory of the saphenous nerve was defined on the basis of perfusion images taken 4 days after transection or perineural capsaicin treatment of the saphenous nerve. In each experiment the color-coded perfusion images showing the maximal vasodilatatory response were selected to process further with the ImagePro 6.2 image analysis software (MediaCybernetics, Rockville, MD, USA). Functional reinnervation was evaluated by measuring the changes in the intensity of the vasodilatatory responses registered in the saphenous and sciatic areas. The color-coded perfusion images recorded at the time of maximal vasodilation were used to determine the proportion of innervated and denervated areas. After subtracting background pixel values by normalizing all pixel data with the 90 percentile of the maximal response, color segmentation was applied on the perfusion image to demarcate and separate areas showing no or minimal vasodilation from those exhibiting large (or maximal) perfusion increases. To perform this segmentation, the 20 percentile of the lateral (intact) side was calculated to set a threshold value for each individual images. Pixels with intensity values lower than the 20 percentile of the intact side were considered as denervated. This step was followed by the generation of a binary mask representing the size and topography of denervated cutaneous areas corresponding to the innervation area of the saphenous nerve. Functional

reinnervation was characterized by measuring the intensity of the vasodilatatory response in the saphenous skin area as defined above.

4. **Immunohistochemical demonstration of the sensory innervation of the skin**

After performing transcardial perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4), the dorsal hairy skin of the hind paw was excised and postfixed in the same fixative for 2 hours. The postfixed tissue was then placed into a phosphate-buffered 30% sucrose solution for cryoprotection. Serial sections of dorsal hind paw skin in 20 μm thickness were prepared with a cryostat. Sections were mounted on gelatin-coated glass slides then rinsed twice in phosphate-buffered saline (PBS) and incubated overnight with the primary antibodies with 1% Triton X100 added. Antibodies against β-tubulin III (mouse monoclonal, 1:500, Sigma-Aldrich GmbH, St. Louis, MO, USA) and CGRP (rabbit polyclonal, 1:4500, Sigma-Aldrich GmbH, St. Louis, MO, USA) were used to label all the nerve fibers and the peptidergic fibers, respectively. After rinsing in PBS the sections were incubated for 2 hours with the secondary antibodies (donkey anti-rabbit and anti-mouse IgGs labeled with CY3 and DL488, respectively, 1:500) diluted in PBS containing 1% TritonX100. The sections were rinsed in PBS then the slides were covered with ProLong Gold Antifade Mountant with DAPI (Invitrogen, ThermoFisher Scientific, Waltham, MA USA).
The sections were examined under a Zeiss confocal fluorescence microscope. Z-stack image series with tile scan mode were obtained.

5. **Visualization of neurogenic plasma extravasation and vascular permeability changes by vascular labeling technique**

To study the morphological distribution of increased permeability in both the denervated and normally innervated skin areas the vascular labeling technique was used. A 1% solution of colloidal silver (Sigma-Aldrich, St. Louis, MO, USA, 50 mg/kg b.w.) was administered intravenously to the tail vein at the time of epicutaneous application of 5% mustard oil onto the dorsal surface of the hind feet. Skin areas with increased vascular permeability due to intact innervation showed brownish color while the reaction was absent in denervated skin regions. Photographs were taken for further analysis of the ratio of denervated and innervated skin areas

using the Image Pro software. For long survival 2 weeks later and for short survival 30 minutes later the animals were sacrificed and were perfused transcardially with 4% paraformaldehyde. Skin of the hind paws were removed. Small sections were excised, fixed in methanol, cleared with xylene and mounted in Canada balsam for light microscopic examination. We have also performed a series of 1% colloidal silver administration on an experimental group (n = 3) for the purpose of following up the territorial changes of the two cutaneous nerves.

6. Statistics

Data represent means ± S.E.M. of 5–9 independent measurements. For statistical comparisons of the mustard oil-induced vasodilatatory responses one-way ANOVA test was run followed by multiple comparisons using the Dunett's post-hoc analysis or Fisher's Least Significant Difference test. In all groups, normality was proved by the Shapiro-Wilk test and homogeneity of variances was confirmed by Levene's test in advance of performing ANOVA. Statistical analysis was performed by using Statistica 6.4 software (Dell Inc., Tulsa, OK, U.S.A.).

RESULTS

1. Characteristics of the mustard oil-induced vascular reactions

Perfusion pattern of the dorsal skin surface of the hind paws was shown to be largely uniform as scanned by laser Doppler perfusion imager. The coefficients of variance of the basal perfusion values recorded on subsequent perfusion images representing the basal blood perfusion of the hind paw were 0.043 ± 0.28 (medial aspect) and 0.054 ± 0.027 (lateral aspect) with no significant difference between the two sides ($p = 0.19$). Epicutaneous application of 5% mustard oil on the dorsal surface of hind paws of intact rats results in significant, immediate vasodilation affecting the entire dorsal surface of the hind paws which peaked in a few minutes (2–4 minutes). Baseline skin perfusion recovered in 15–20 minutes after the onset of the vasodilation. Maximal increase of the perfusion was 80–85% on the whole site of application and there were no difference between the medial and lateral side. Vasodilation could be elicited repeatedly resulting in similar perfusion patterns but with lower maximal changes.

2. Effect of nerve transection and capsaicin-induced selective chemodenervation on mustard oil-induced vasodilatation

There was no difference observed in the basal blood flow values in the denervated (saphenous) and the intact (lateral) skin areas of the dorsal skin of the hindpaw. However, the magnitude of the mustard oil-induced vasodilatatory response was significantly declined in the medial, denervated area 3 days after treatment of the saphenous nerve, as compared with the lateral territory of the intact sciatic nerve. This was the case both after transection and selective chemodenervation of the saphenous nerve. Both treatments resulted in a relative perfusion change of the lateral, untreated side of the hind paw of about 80–100%. By contrast, following transection of the saphenous nerve, the medial, completely denervated side showed a marked difference with maximal relative change of $32 \pm 4\%$. Perineural capsaicin treatment of the saphenous nerve produced changes in the skin blood flow similar to nerve transection with markedly reduced mustard oil-induced vasodilatatory reaction ($39.03 \pm 16.38\%$) compared to the intact lateral side. Time course of the vasodilatatory reaction in the intact lateral areas has been shown being similar to the untreated, control group. Results show that both the transection and perineural capsaicin treatment resulted in significant decrease of sensory neurogenic vasodilation in the treated, medial skin in the acute postoperative phase indicating the impairment of the efferent vasodilatatory function of the peptidergic afferent nerves serving the medial part of the rat dorsal hind paw.

3. Reinnervation of the denervated skin following nerve transection

The vasodilatatory response showed a marked progressive recovery towards control values initially in the saphenous skin area immediately bordering the innervation territory of the sciatic nerve 20 days after axotomy. Spreading of the area displaying mustard oil-induced vasodilation gradually continued toward the medial sites and about 4 weeks were required to reach the topography of the vasodilatatory response similar to that observed before denervation. In parallel, maximal values of the mustard oil-induced vasodilation in both medial and lateral sides progressively become similar. It was supposed that the origin of the nerve fibers reinnervated the medial aspect of the dorsal hindpaw derived from the adjacent sciatic nerve. To verify this suggestion, the sciatic nerve was subjected to local, perineural treatment with capsaicin to defunctionalize chemosensitive afferent nerves running in the sciatic nerve trunk. This defunctionalization resulted in the absence of sensory neurogenic vasodilatatory response 3-4 days after the capsaicin treatment not just in the territory of the sciatic nerve but also in the

medial aspect of the hindpaw originally served by the saphenous nerve. These results indicate that recovery of the vasodilatatory response may be attributed to sciatic afferents through collateral sprouting.

4. **Reinnervation of the chemodenervated skin by collateral sprouting occurs only after transection of the capsaicin-treated nerve**

Importantly, a tendency for recovery of the vasodilatatory response was not observed after perineural capsaicin treatment even on the 7th postoperative week. Maximal perfusion changes of the lateral, intact side were significantly higher than that of the medial side during the follow-up period of 7 weeks. The proportion of denervated skin areas showed no significant change during this long follow-up period after perineural capsaicin which may indicate that degenerative processes underlie this permanent reduction of the vasodilatatory reactions. It was assumed that nerve fibers not affected by perineural capsaicin treatment may persist in the capsaicin-treated nerve trunk and within its innervation skin territory and these fibers may hinder collateral ingrowth of intact afferent nerve fibers from the neighboring sciatic cutaneous innervation territory. To confirm this theory, we transected the right saphenous nerve 7 weeks after perineural capsaicin treatment and observed the subsequently induced vasodilatatory reactions. Evaluation of perfusion images revealed that 2–4 weeks following the transection of the previously capsaicin treated saphenous nerve the proportion of the denervated skin areas gradually decreased while in the chemodenervated area, the proportion of reinnervated areas gradually increased and peaked 4 weeks later at around 40%. To further support the assumption of collateral reinnervatory process, the sciatic nerve was treated perineurally with capsaicin that abolished the recovered vasodilatatory response within the area of the capsaicin-treated saphenous nerve.

To confirm the results obtained by laser Doppler imaging, mustard oil-induced vascular labeling technique was applied and the topographical distribution of the labeling pattern was repeatedly assessed. Intravenous injection of colloidal silver followed by the application of mustard oil onto the skin surface a clear-cut vascular labeling of permeable small subepidermal venules is produced in skin areas of intact sensory innervation but could not be elicited in denervated skin. The results obtained with vascular labeling and scanning laser Doppler imaging were essentially similar. Following perineural capsaicin treatment and subsequent transection of the saphenous nerve photograph series were captured at different time intervals to visually follow-up the size and topography of the denervated skin regions and the gradual

recovery of the vascular labeling on the previously denervated cutaneous areas. These areas were continuously measured to gain quantitative data. Data showed marked reinnervation of the chemodenervated skin following transection of the capsaicin-treated saphenous nerve. It may be observed that the proportion of the chemodenervated skin area gradually decreased with simultaneous gradual increase in the proportion of reinnervated area in the chemodenervated skin after the transection of the capsaicin-treated saphenous nerve.

5. Immunohistochemical study of degeneration and regeneration of cutaneous nerves after peripheral nerve lesions

Tubulin III- and CGRP-immunohistochemistry can be applied for the demonstration of nerve fibers innervating the epidermis, hair follicles and small blood vessels of the skin. Tubulin III is a reliable general marker of all types of nerve fibers in a variety of tissues, whereas CGRP is a specific marker of the major population of the peptidergic afferent nerves. Intact innervated skin shows a rich network of solitaire fibers or fiber bundles of tubulin- and/or CGRP-immunoreactive nerve fibers in all layers of the dermis and hypodermis. Tubulin positive single nerve fibers showing tubulin immunoreactivity are also abundant, but only a fraction of them exhibit also CGRP positivity.

Nerve transection resulted in the loss of nerve fibers in the denervated saphenous skin areas 4 days after the surgery. In contrast, the innervation territory of the intact sciatic nerve was similar to control. The first histological signs of reinnervation were detected 14 days after saphenous nerve transection, as few silver-labeled blood vessels could be observed in the previously denervated skin area with the re-appearance of some tubulin- and CGRP-immunopositive nerve fibers in the epidermal and subepidermal layers. This restituted innervation was completely lost after perineural capsaicin treatment of the sciatic nerve by the end of the experiments supporting the findings of the functional studies and confirming the collateral sprouting mechanism.

Unlike nerve transection, selective chemodenervation with perineural capsaicin treatment did not deplete completely the nerve fibers in the dermis and hypodermis. Residual fibers positive for tubulin but not for CGRP can be also observed in the epidermis 4 days after perineural capsaicin treatment. In contrast with nerve transection, the CGRP immunoreactivity did not recover, but remained absent for the whole 7-week follow-up period until the transection of the capsaicin-treated nerve. Two weeks following the transection of the previously capsaicin-treated saphenous nerve a few CGRP-positive fibers and vascular labeling with colloidal silver

reappeared on the medial side originally innervated by the transected saphenous nerve. Restitution of CGRP-immunoreactivity and vascular labeling can not be achieved through regenerative growth of the saphenous nerve as it was ligated thus the collateral sprouting of the neighbouring intact nerve fibers may be responsible for restoring the innervation of the denervated skin area. Reappearance of vascular labeling indicates not just morphological but functional recovery of the previously denervated cutaneous regions.

DISCUSSION

Information on the functional status of cutaneous nerves is of fundamental clinical and experimental importance in assessing degenerative and regenerative processes of sensory nerves and the progression of diseases affecting the peripheral nervous system. Different techniques may be applied to study sensory functions of skin nerves involved in the sensation of specific modalities, such as touch, vibration, cold, warm, itch and pain. Although sensory functions of the skin and, after selective denervation procedures, cutaneous innervation territories of peripheral nerves can be assessed with these methods, these techniques are neither accurate nor objective. These technical difficulties come even more to the front if longitudinal studies are to be performed.

The visualization of the neurogenic inflammatory response for objective study of the function and spatial distribution of a particular population of peptidergic cutaneous nociceptors has been introduced in earlier studies performed in our laboratory. In the denervated skin, neurogenic plasma extravasation cannot be evoked with mustard oil and, therefore innervated and denervated skin areas can be clearly defined by the presence and absence, respectively, of Evans blue coloration of the skin. The spatial distribution of small blood vessels (venules) of enhanced vascular permeability following the cutaneous administration of mustard oil can also be visualized by using the vascular labeling technique. Upon stimulation of nociceptive nerve endings with mustard oil, colloidal silver accumulates in the wall of the permeable blood vessels which can be easily visualized under the light microscope in transparent preparations. The innervated territories of the skin can also be seen with the naked eye and photographed owing to the brownish discoloration of the skin.

In the present Thesis we have applied a novel approach to demonstrate the cutaneous innervation territories of hindlimb nerves utilizing the visualization of mustard oil-induced sensory neurogenic vasodilatation by using the non-invasive LDI technique. These functional

studies were combined with immunhistochemical demonstration of cutaneous peptidergic nerves in skin areas identified by the presence or absence of mustard oil-induced vascular labeling.

In the skin the examination of neurogenic vasodilatatory response by laser Doppler imaging (LDI) technique, similarly to previous techniques (e.g Evans blue extravasation, vascular labeling) is a reliable method to assess functional integrity of chemosensitive cutaneous nerve fibers. This method offered appropriate spatial and temporal resolution, therefore it is suitable to explore the time course and topographic pattern of the intensity changes of cutaneous blood perfusion caused by regional circulatory reactions evoked by the TRPA1 agonist mustard oil. Since cutaneous sensory denervation caused reduction or complete elimination of the neurogenic inflammatory reaction LDI has been proved to be suitable to demarcate the denervated skin region shortly after the complete transection of peripheral nerve or perineural capsaicin treatment. Compared to previously applied techniques, however, LDI appeared to be a reliable and suitable method for longitudinal observation of reinnervation processes in the skin of the same individual following peripheral nerve injuries. Similar neurogenic vasodilation can be observed also in humans, therefore this technique may allow the objective and non-invasive clinical assessment of axon regeneration and reinnervation after peripheral nerve injuries.

Transection and ligation of the saphenous nerve resulted in a dramatic, although not complete reduction of mustard oil-induced vasodilatation in the denervated skin 3–4 days after the surgery. Signs of reinnervation were first observed at the 3rd postoperative week with a gradual and time-dependent reappearance of the vasodilatatory response in the denervated skin as measured by repeated assessments of mustard oil-induced vasodilation. After approximately 3-4 weeks the topography and the intensity of the vasodilatatory response were similar to the pre-surgical condition. This time-course of functional recovery is consistent with observations of earlier studies utilizing behavioral testing or the Evans blue technique to examine functional cutaneous nerve regeneration. Upon perineural capsaicin treatment of the saphenous nerve the mustard oil-induced neurogenic vasodilatatory response has appeared to be completely and permanently lost in the skin area served by the affected nerve. During the 7-week follow-up period, we did not observe any sign of functional recovery of the neurogenic vasodilatatory response. This finding agrees with previous observations demonstrating a permanent loss of C-fiber afferents in the peripheral nerves or cutaneous skin regions affected by the capsaicin-induced selective chemodenervation. This suggests that neither the regenerative regrowth of

the afferents in the treated saphenous nerve nor the collateral sprouting of the intact neighboring sciatic afferents have reinnervated the previously chemodenervated skin region. However, following the transection and ligation of the capsaicin-treated saphenous nerve, the mustard oil-induced vasodilatatory response showed a gradual and significant recovery in both intensity and spatial extent similarly to that observed after the transection of the saphenous nerve. Since the ligation of the transected saphenous nerve prevented its regeneration, the recovery of neurogenic vascular reactions was attributed to reinnervation of the denervated area through collateral sprouting, i.e. the invasion of adjacent intact sciatic afferents into the saphenous skin territory. This notion was supported by immunohistochemical demonstration of CGRP positive fibers in the innervation area of the saphenous nerve. In addition, perineural capsaicin treatment of the sciatic nerve after the reinnervation of the saphenous area resulted in the elimination of the vasodilatatory response both in the sciatic and in the saphenous skin territories supporting the assumption that reinnervation has been achieved through the collateral sprouting mechanism. These observations were confirmed by demonstrating the recovery of the mustard-oil induced vascular labeling in the previously chemodenervated skin area shortly after the complete denervation of the saphenous area by surgical transection of the capsaicin treated saphenous nerve.

These observations suggest novel pathophysiological/neuroplastic changes which commence after chemodenervation of the skin by perineural treatment with capsaicin. The phenomenon of inhibition of reinnervation of the denervated skin by collateral sprouting of adjacent intact nerves may be referred to as collateral inhibition of nerve regeneration. The findings also suggest a permissive effect of transection of the capsaicin-treated (saphenous) nerve promoting collateral sprouting. The exact mechanism of this permissive effect is not known at present. We assume that non-degenerating unmyelinated and myelinated nerve fibers which persist in the chemodenervated skin may inhibit the sprouting of adjacent intact sciatic nerve fibers into the denervated skin area. Contribution of glial cells of the partially denervated peripheral nerves in the regulation of the collateral sprouting-based reinnervation process has also been proposed. Similar processes resulting in failure of regeneration may be involved in certain human neuropathies which affect only subpopulations of afferent nerves.

CONCLUSION

In human and rodent skin, the application of mustard oil elicits sensory neurogenic vasodilatation in the intact but not in the denervated skin. Measuring the mustard oil-induced sensory vasodilatatory response by repeated scanning Laser Doppler perfusion imaging is a reliable technique to assess the functional integrity and the regenerative capacity of cutaneous nociceptive nerves. The method is suitable for the longitudinal non invasive follow-up of changes in both the topographical distribution and the intensity of the vasodilatatory response following peripheral nerve injuries, such as nerve transection or chemodenervation.

By using this novel experimental approach, we confirmed the difference in regenerative tendencies following surgical and chemical denervation of peripheral sensory nerves consistently with previous observations. After surgical denervation of saphenous nerve partial reinnervation is achieved via collateral sprouting of adjacent intact sciatic chemosensitive afferents. By contrast, such collateral sprouting could not be observed in skin areas chemodenervated by perineural capsaicin treatment. An important observation of the present study is that transection and ligation of the previously capsaicin treated saphenous nerve allows for the intact sciatic afferents to invade into the denervated skin areas through collateral sprouting.

These findings suggest a neuroplastic phenomenon of collateral inhibition of nerve regeneration that may be attributed to an inhibitory effect of capsaicin-insensitive sensory nerves persisting in the chemodenervated cutaneous regions.

Further studies to provide clues for the exact mechanism(s) of this permissive conditioning lesion effect promoting collateral sprouting may expands our knowledge about the axonal regenerative processes under neuropathic conditions characterized by the partial sparing of nerve fibers and target innervation.

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