Perineural capsaicin treatment attenuates reactive hyperaemia in the rat skin

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Abstract

The neural mechanisms of reactive cutaneous hyperaemia were studied by using a novel experimental approach: the simultaneous measurement of cutaneous blood flow by laser-Doppler flowmetry in adjacent innervated and chemodenervated skin regions of the rat hindpaw served by the same artery. Transient occlusion of the femoral artery (0.5–6 min) resulted in reactive hyperaemia that was greatly reduced in the chemodenervated region. After 3 min arterial occlusion, peak cutaneous blood flow was 109 ± 13% vs. 53 ± 7%* (% change from baseline, n = 11, *P < 0.05), and the total hyperaemic response was 110 ± 21 vs. 52 ± 12* (arbitrary perfusion units) in intact vs. chemodenervated skin regions, respectively. The findings provide clear evidence for the involvement of peptidergic capsaicin-sensitive afferent nerves in the mechanism of reactive cutaneous hyperaemia.

The capsaicin-sensitive primary sensory neurones comprise a morphologically and pharmacologically well-defined division of the somatovisceral afferent system [8]. Most of these neurones are polymodal nociceptors, and are involved in local regulatory sensory efferent functions by the release of neuropeptides from their peripheral endings. In the skin, these special afferents play a key role in the mechanisms of antidromic vasodilation and neurogenic plasma extravasation. The involvement of capsaicin-sensitive afferent nerves in various cutaneous reactions is well established. There is also circumstantial evidence of the participation of these particular afferent nerves in the mechanism of postocclusive cutaneous reactive hyperaemia [13,14]. Reactive hyperaemia seems to involve neurogenic, myogenic and metabolic components, but its exact mechanism is still unclear. The contribution of capsaicin-sensitive nerves to the mechanism of various vascular reactions has been reliably demonstrated by utilizing the selective neurotoxic effect of systemically administered capsaicin (capsaicin desensitization) [9,11]. Indeed, a previous study showed that systemic capsaicin treatment results in decreased reactive hyperaemia in the rat hindlimb [14].

In the present study, we investigated the participation of capsaicin-sensitive afferent neurones in the mediation of cutaneous reactive hyperaemia in the rat by using perineural capsaicin treatment, a technique that has several advantages over systemic capsaicin administration. The local application of capsaicin onto a peripheral nerve results in a highly selective and long-lasting functional impairment of the capsaicin-sensitive afferent nerves [6,10,12]. In contrast with the systemic administration of capsaicin, the perineural application of capsaicin affects only those afferents which run in the treated nerve. Indeed, by choosing appropriate experimental conditions, it is possible to study the effects of transient arterial occlusion on the cutaneous blood flow in adjacent normally innervated and chemodenervated skin areas, respectively. This offers a unique possibility for study of the effects of selective sensory denervation on the reactive hyperaemia in neighbouring skin regions supplied by the same artery, which eliminates various problems that may arise if results obtained in different animals are compared. In the rat, the adjacent medial and lateral parts of the hindpaw served by the same artery were used for the study.

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of the dorsal skin of the hindpaw are innervated by peptidergic sensory fibres of the saphenous and the sciatic nerve, respectively [1]. In the present study, laser-Doppler flowmetry (LDF) was used to examine the effects of perineural capsaicin treatment on the cutaneous blood flow changes elicited by transient arterial occlusion in the rat hindpaw skin.

Male Wistar rats (240–300 g, \(n = 6\)) were anaesthetized with chloral hydrate (400 mg/kg, i.p.; Reanal, Hungary). The sciatic nerves were exposed high in the thigh, and small pieces of gelfoam (Gelaspon, Chauvin-Ankerpharm, Germany) moistened with capsaicin (1%, 100 \(\mu\)l; Fluka, Germany) or an equivalent amount of the vehicle (6% ethanol, 8% Tween-80 in saline) were placed on the right and left nerves, respectively. After 20 min, the gelfoam pieces were removed, the wounds were closed and the rats were returned to the animal house. Seven to 10 days later, the rats were anaesthetized again (chloral hydrate, 400 mg/kg, i.p.), a polyethylene cannula was placed into the trachea through a small incision and the animals were allowed to breathe room air spontaneously. Body temperature was kept constant (37–38 °C) with the aid of a heating pad. The femoral artery was exposed and isolated where it appears under the inguinal ligament. A plastic thread was placed underneath the artery, allowing transient occlusion of the vessel. Cutaneous blood flow was monitored with a 2-channel LDF (Periflux 4001, Sweden). The LDF flow probes were placed over the medial (served by the saphenous nerve) and the lateral (served by the sciatic nerve) surface of the dorsal skin of the hindpaws, respectively. LDF data were collected online with an IBM computer.

After stable baseline values had been attained, reversible arterial occlusion was performed for 0.5, 1, 3 and 6 min by gently lifting the thread (\(n = 5, 5, 11, \) and 8, respectively, four to six trials in each animal). Data were collected for 6 min after completion of the occlusion period. Individual trials were separated by a waiting period of at least 20 min to minimize possible effects of previous trials on reactive hyperaemia. Since no such effects were observed, all data were combined as presented. Then, 3 min arterial occlusion induced reactive hyperaemia (\(n = 6\)) was tested in the vehicle-treated leg. After completion of the blood flow measurements, the rats were given an injection of a 1% solution of Evans blue dye (50 mg/kg, i.v.; Sigma-Aldrich, USA), and the dorsal skin of the hindpaw was painted with mustard oil (5% in liquid paraffin; Sigma) to elicit a neurogenic plasma extravasation response in order to control the effectiveness of chemodenervation. Twenty minutes later the deeply anaesthetized animals were killed by decapitation, bled, and skin samples involving the measurement sites were removed (\(n = 5\)) and their dye content was determined by spectrofotometry [10,11]. All data are expressed as mean ± SEM. Student’s \(t\)-test was performed to detect significant differences between the blood flow and Evans blue dye content data of intact and chemodenervated skin, and one-way analysis of variance was used to compare the reactive hyperaemia of different occlusion periods.

Transient occlusion of the femoral artery resulted in a marked decrease in cutaneous blood flow. Arterial occlusion for 0.5, 1, 3, and 6 min reduced cutaneous blood flow similarly to 36 ± 8, 41 ± 8, 28 ± 2, and 31 ± 3%, and 43 ± 9, 44 ± 13, 29 ± 4, and 36 ± 5% of baseline perfusion in the medial, intact and in the lateral, chemodenervated skin areas, respectively. Release of the occluded artery restored cutaneous blood flow quickly to give rise to reactive hyperaemia. There were no significant differences in the dynamics and the duration of reactive hyperaemia between the intact and chemodenervated skin regions. The maximum increases in cutaneous blood flow during reactive hyperaemia were determined as percentage increases in perfusion compared to baseline levels. The peak cutaneous blood flow appeared to be independent of the duration of the preceding arterial occlusion: it was above the baseline level by approximately 90–110% after 1–6 min of ischaemia in the intact medial skin (Fig. 1). However, the peak cutaneous blood flow values were significantly attenuated, by 40–50%, in the lateral, chemodenervated skin as compared with the medial, intact skin after 1–6 min of ischaemia (Fig. 1). The total increase in blood flow during reactive hyperaemia was determined by calculating the area under the LDF signal-time curve, and expressed in arbitrary perfusion units. The total hyperaemic response (in arbitrary perfusion units) after 0.5, 1, 3, and 6 min occlusion was 10 ± 2 vs. 5 ± 1*, 38 ± 14 vs. 15 ± 4*, 110 ± 21 vs. 52 ± 12*, and 128 ± 18 vs. 77 ± 20* in intact vs. chemodenervated areas (*\(P < 0.05\)), respectively. Thus, similarly to the peak

Fig. 1. Effects of perineural capsaicin treatment (chemodenervation) on peak (maximal) postocclusive cutaneous reactive hyperaemia in medial (intact) and lateral (chemodenervated) areas of the rat dorsal hindpaw skin. Peak cutaneous blood flow is plotted as a percentage increase from the baseline taken for 1 min preceding the femoral artery occlusion. The peak blood flow did not vary much with the duration of occlusion, however, there was a striking difference between peak blood flow values of intact and chemodenervated areas; it was significantly depressed by about 50% in chemodenervated skin (*\(P < 0.05\), \(n = 5, 5, 11, \) and 8, respectively).
cutaneous blood flow responses, the total cutaneous blood flow responses were also decreased by about 50% in the chemodenervated skin, as compared with the adjacent normally innervated skin (Fig. 2). In contrast, the medial and lateral skin areas gave similar reactive hyperaemic responses in the vehicle-treated leg (Fig. 2).

In the normally innervated skin, a deep blue coloration due to the extravasation of Evans blue dye bound to serum albumin indicated the development of the neurogenic inflammatory response. A lack of such coloration upon the application of mustard oil indicated the effectiveness of perineural capsaicin treatment [10] and verified the proper placement of the LDF probes. Determination of the Evans blue content of skin samples obtained from skin areas innervated by intact (vehicle-treated) and capsaicin-treated nerves demonstrated an almost complete lack of dye accumulation in the chemodenervated skin after the application of mustard oil (innervated: 23.3 ± 1.2 µg/100 mg; chemodenervated: 2.1 ± 0.9* µg/100 mg; n = 5, *P < 0.05). In agreement with previous observations, these data indicate that perineural capsaicin treatment resulted in a practically complete depletion of sensory neuropeptides involved in the mechanism of neurogenic plasma extravasation and neurogenic sensory vasodilation [1,6,7,16,17].

The present findings provide evidence that capsaicin-sensitive afferent nerves are intimately involved in the mechanism of postocclusive cutaneous reactive hyperaemia. The novel experimental model utilized in this study is especially suitable for demonstration of the role of capsaicin-sensitive afferent nerves in the modulation of the vascular reactions of the affected tissues: direct simultaneous comparisons of the vascular reactions occurring in the innervated and in the chemodenervated skin areas are performed in the same animal, on adjacent skin regions receiving their vascular supply from the same arterial system. This experimental paradigm ensures that differences in vascular responses observed in intact and denervated skin regions may be attributed to the transient arterial occlusion and do not result from differences in major physiological parameters (general condition) of the animal, the level of ischaemia induced by arterial occlusion, the systemic blood pressure, the degree of anaesthesia, the ambient temperature or other experimental variables. Hence, using a non-invasive technique for the measurement of cutaneous blood flow, the present study demonstrates that perineural capsaicin treatment affects the basic vasoregulatory mechanisms of chemodenervated tissues, such as postocclusive vasodilation.

The data reveal that perineural capsaicin severely depresses the magnitude of the reactive hyperaemia response (both the peak response and total increase in cutaneous blood flow) but fails to affect the temporal characteristics (onset or duration) of the response. These observations suggest that, although the accumulation of vasoactive tissue metabolites during the ischaemic period may play a decisive role in the initiation and maintenance of reactive hyperaemia, capsaicin-sensitive afferent nerves significantly amplify the response. Hence, elimination of this particular population of polymodal nociceptive nerve fibres significantly reduces the hyperaemic responses, by about 50%, almost irrespectively of the duration of the ischaemia.

The exact mechanism(s) by which perineural treatment with capsaicin reduces cutaneous reactive hyperaemia is at present unclear. Perineural application of capsaicin results in long-lasting chemical and thermal analgesia and abolition of the neurogenic inflammatory response associated with and probably caused by substantial reductions in substance P and calcitonin gene-related peptide (CGRP) immunoreactivity in the related skin areas [2]. The available morphological, functional and electrophysiological evidence indicates that perineural treatment with capsaicin is a highly selective technique for the elimination of peptidergic capsaicin-sensitive polymodal nociceptive afferent fibres [2,4,6,13]. CGRP has been shown to mediate the vasodilatory responses elicited by orthodromic or antidromic stimulation of capsaicin-sensitive afferent nerves [3,15]. Therefore, the most likely explanation of the present findings is that chemodenervation by capsaicin abolishes the augmentation of the reactive hyperaemia response due to the depletion/loss of capsaicin-sensitive C-fibre afferent nerves containing CGRP. It can be suggested that, in the intact skin, tissue metabolites accumulating during ischaemia and an increase in [H+] may stimulate peptidergic sensory nerve endings, resulting in a consequent

![Fig. 2. Decreased postocclusive reactive hyperaemia in skin chemodenervated by perineural capsaicin treatment. Total reactive hyperaemia was determined by calculating the area under the laser-Doppler flow signal-time curve in arbitrary perfusion units. Data were plotted as percentage decreases in total reactive hyperaemia in the lateral skin region compared to the corresponding response of the medial area. In the vehicle-treated controls (n = 6), there was no difference in reactive hyperaemia induced by 3 min femoral artery occlusion between the medial (intact) and lateral (sham-chemodenervated) skin areas. In contrast, in all animals subjected to perineural capsaicin treatment, there was a consistent 40–50% decrease in cutaneous perfusion, chemodenervated skin areas irrespectively of the duration of ischaemia (*P < 0.05, n = 5, 5, 11, and 8, respectively).](image-url)
release of vasodilatory sensory neuropeptides, including CGRP, which augments the reactive hyperaemic response. This is supported by previous findings that CGRP is released by lactic acid and low pH from capsaicin-sensitive afferent nerves under ischaemic conditions [5]. Increased clearance of tissue metabolites that accumulate during ischaemia and the consequent restoration of the local metabolism, normal sensory function and blood flow may underlie the biological significance of the sensory nerve-mediated augmentation of postocclusive reactive hyperaemia. Additionally, the present findings provide further evidence that capsaicin-sensitive afferent nerves may be of significance in the pathogenesis of vascular alterations observed in various diseases associated with sensory neuropathy.

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