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Research article

Involvement of oxidative stress in increased peripheral nerve firing during spontaneous dysesthesia in a mouse model of ischemia-reperfusion

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HIGHLIGHTS

- Transient ischemia-reperfusion in the hand and foot elicits spontaneous dysesthesia.
- We established a mouse model of hind-paw transient ischemic-reperfusion.
- In this model, peripheral nerve activity and lipid peroxidation were increased.
- An antioxidant reagent countered both the nerve activity and lipid peroxidation.
- Both oxidative stress and nerve activity may contribute to peripheral dysesthesia.

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ABSTRACT

Transient ischemia-reperfusion in the hand and foot elicits spontaneous dysesthesia. However, the mechanisms by which this occurs are not completely understood. The objectives of this study were to examine peripheral neural activity related to spontaneous dysesthesia in a mouse model of hind-paw transient ischemic-reperfusion and to investigate the involvement of oxidative stress in this neural activity. The femoral artery and vein were interrupted for 10 min using tourniquet pressure, before the tourniquet was removed to allow reperfusion of the hind paw. Neural activity in the saphenous nerve was recorded during both ischemia and reperfusion. In both the ischemic phase and the reperfusion phase, the frequency of saphenous nerve firing was significantly increased compared to baseline. The antioxidant agent *N*-acetyl-L-cysteine inhibited significantly the firing of the saphenous nerve in both the maximum and minimum activity periods during ischemia, and in the maximum activity state after reperfusion percentage inhibition being approximately 68%, 60%, and 58%, respectively. In the reperfusion phase, the production of 4-hydroxy-2-noneal, a major product of endogenous lipid peroxidation, was significantly increased in the plantar skin, and this was inhibited by *N*-acetyl-L-cysteine. In the ischemic phase, a similar trend was observed. These results suggest that an increase in peripheral nerve activity related to oxidative stress may be involved in the spontaneous dysesthesia induced by transient ischemia-reperfusion.

1. Introduction

In reperfusion after transient ischemia, most people experience unpleasant and abnormal sensations, such as tingling, pricking, and electric-shock-like sensations. This occurs in the limbs after sitting down in the Japanese formal style of 'seiza', and in one's fingertips after sleeping with one's arm crooked under one's head. A common cause is leaning or lying awkwardly on the extremities,

Abbreviations: 4-HNE, 4-hydroxy-2-noneal; H_2O_2, hydrogen peroxide; NAC, *N*-acetyl-L-cysteine; PVDF, polyvinylidene difluoride; ROS, reactive oxygen species; SEM, standard error of the mean.

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which either presses against the nerves or reduces the blood supply beyond the site of compression.

These unpleasant sensations, whether spontaneous or evoked, are termed dysesthesia. In the clinic, patients with diabetes and those receiving chemotherapy complain of dysesthesia that is similar to the experiences of ischemia-reperfusion [1,2]. With the neuropathic consequences of these conditions, peripheral blood flow is decreased [3,4], which suggests the possibility that changes in peripheral blood flow might contribute to dysesthesia. As dysesthesia contributes to various neuropathic pain conditions, effective treatment of dysesthesia is important for the improvement of neuropathy patients' quality of life. However, determining the mechanisms underlying dysesthesia has been problematic. Even in patients suffering from the same type of ischemic insult or neuropathic condition, the manifestations of the dysesthesia may be different due to differences in the mental, physical, or environmental backgrounds of these patients [5–7]. In healthy subjects, abnormal sensations can be induced by ischemia and reperfusion or tonic muscle contraction of the limbs [8], but there is a limit to the detailed molecular mechanism analysis that can be done in human experiments. In spite of this, few suitable animal models of spontaneous dysesthesia have been developed. Although ischemia-reperfusion of the tail in rats using tourniquet pressure has been shown to cause thermal and mechanical hyperalgesia [9,10], spontaneous dysesthesia has not been evaluated in this model. We recently demonstrated that reperfusion after ischemia in the mouse hind paw markedly provokes dysesthesia-related spontaneous licking of the treated site [11]. However, objective evaluation of the dysesthesia-related response was not performed. Therefore, in this study, as an index of dysesthesia, we examined the peripheral neural activity in a mouse model of transient ischemic-reperfusion of the hind paw using electrophysiological methods. Specifically, we aimed to determine dysesthesia-associated neural activity due solely to ischemia-reperfusion without direct compression of the peripheral nerve. In this study, we recorded the activity of the saphenous nerve, which innervates the dorsal surface of the thumb-side of the paw [12] and is composed entirely of sensory neurons, including both myelinated and unmyelinated nerve fibers [13].

We have previously shown that oxidative stress and Aδ-fiber-evoked responses may be the cause of peripheral ischemia-associated dysesthesia [11]. Transient ischemic injury generates reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2) due to oxygen rapidly returning to the ischemic tissue [14]. However, no studies have examined the changes in peripheral nerve activity and oxidative stress in ischemia–reperfusion-induced dysesthesia. Therefore, in this study, we also investigated the involvement of oxidative stress in dysesthesia-related nerve activity.

2. Materials and methods

2.1. Animals

Male 6-week-old C57BL/6 mice (Japan SLC, Inc., Hamamatsu, Japan and Charles River Laboratories, Japan) were used. They were kept in rooms of controlled temperature (20–26 °C) and humidity (30%–70%) and 7:00a.m.–7:00p.m. alternated light-dark cycles. Food and water were freely available. Experiments were conducted with the approval of the Animal Care Committee of the University of Toyama, and Institutional Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation (Osaka, Japan), and in accordance with the guidelines for the investigation of experimental pain in animals published by the International Association for the Study of Pain [15].

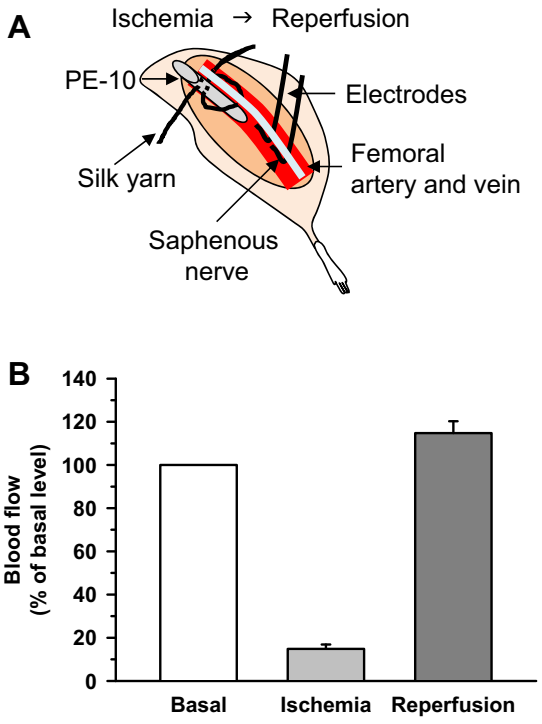

Fig. 1. Changes in plantar skin blood flow induced by ischemia-reperfusion in the hind paw. (A) Schematic of the left limb and hind paw, showing the placement of the tourniquet and recording electrodes. Polyethylene (PE10) tubing was placed alongside the femoral blood vessels for support, and the vessels and tubing were firmly compressed by a silk yarn tourniquet for 10 min to induce ischemia. The silk yarn was cut to allow reperfusion. (B) The blood flow in the plantar skin during the ischemic and reperfusion phases. The blood flow was measured by a laser Doppler flowmeter, and the measurements in the ischemic and reperfusion phases were normalized to the basal level (set to 100%). Data are presented as the mean and SEM of 14 animals.

2.2. Agents

N-Acetyl-L-cysteine (NAC, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in physiological saline and injected intraperitoneally 30 min before the ischemic insult [11]. Saline served as a vehicle control. The volume for administration was 0.1 ml/10 g body weight.

2.3. Compression ischemia of the femoral artery and vein

Mice were anesthetized with sodium pentobarbital (65 mg/kg, intraperitoneally). The skin and connective tissues of the left hind paw were excised to expose the femoral blood vessels. To induce hind-paw ischemia (Fig. 1A), a polyethylene tube (size 10, Igarashi Ika Kogyo Co., Ltd. Tokyo, Japan) was placed alongside the femoral artery and vein for support, and the blood vessels and tubing were firmly compressed by a silk yarn for 10 min with care not to directly touch the saphenous nerve. The silk yarn was then cut to allow reperfusion. The skin blood-flow ratio in the dorsal surface of the paw was measured by a laser Doppler flowmeter (ALF21; Advance Co., Ltd., Tokyo, Japan), which was used equipped with a 1 mW laser emitting at 780 nm. A noncontact probe was used in order to avoid blood flow alterations due to mechanical contact with the skin. In the sham operation, a silk yarn was only passed under the blood vessels without tying.

2.4. Measurement of nerve firing in the saphenous nerve

Fig. 1A represents the design of the experiment. Mice were anesthetized with intraperitoneal sodium pentobarbital (65 mg/kg) and