A Mouse Model of Peripheral Postischemic Dysesthesia: Involvement of Reperfusion-Induced Oxidative Stress and TRPA1 Channel

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ABSTRACT

Peripheral postischemic dysesthesia was examined behaviorally in mice and we investigated the underlying molecular mechanism with a focus on oxidative stress. Hind-paw ischemia was induced by tight compression of the ankle with a rubber band, and reperfusion was achieved by cutting the rubber tourniquet. We found that reperfusion after ischemia markedly provoked licking of the reperfused hind paw, which was significantly inhibited by systemic administration of the antioxidant N-acetyl-L-cysteine and the transient receptor potential (TRP) A1 channel blocker BCTC [N-(4-tert-butylyphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide; I-R, ischemia-reperfusion; ROS, reactive oxygen species; TRP, transient receptor potential].

INTRODUCTION

Most people experience spontaneous thermal paresthesias (abnormal sensations), spontaneous dysesthesias (unpleasant abnormal sensations), such as tingling, pricking, and electric shock-like sensations, and in severe cases mechanical allodynia of the hand and foot after sitting with their legs crossed or sleeping with an arm crooked under their head. Patients with diabetic neuropathy or chemotherapy-induced neuropathy complain of spontaneous dysesthesia (Bastyr et al., 2005; Driessen et al., 2012), and peripheral blood flow is decreased in these neuropathies (Maxfield et al., 1997; Gauchan et al., 2009), raising the possibility that ischemia is responsible for dysesthesia in these pathologic conditions. Understanding the mechanisms of postischemic dysesthesia may provide insight into the cellular and molecular mechanisms underlying dysesthesia in neuropathic conditions, and this requires animal models of peripheral postischemic dysesthesia. Because these sensations are felt superficially in the skin (Merrington and Nathan, 1949), we expected that postischemic dysesthesia would elicit licking behaviors (postischemic licking) in animals. On the basis of this idea, we sought to develop a mouse model of peripheral postischemic dysesthesia.

Myelinated Aβ-fibers are activated by light touches to the skin and discriminate texture and object form, while myelinated Aδ-fibers and unmyelinated C-fibers respond to intense or painful pressure (Delmas et al., 2011). Injury and diseases that affect the function of these fiber subtypes lead to paresthesia and dysesthesia (Scherens et al., 2009). To characterize a mouse model of peripheral postischemic dysesthesia, we examined which types of primary afferents would be involved in postischemic licking.
Peripheral postischemic dysesthesia occurs when blood supply returns (reperfusion) to a previously ischemic tissue. Reperfusion following long-survived ischemia causes severe injury to cells in the target organs (Iida et al., 2009; Klune and Tsung, 2010). Whereas the pathogenesis of ischemia-reperfusion (I-R)–induced tissue injury is not completely understood, I-R injury has been shown to be mediated by the generation of reactive oxygen species (ROS), such as superoxide radical, hydroxyl radical and hydrogen peroxide (H₂O₂), which results from the return of oxygen to the ischemic tissue (Zweier and Talukder, 2006). Several lines of evidence suggest that ROS are involved in mechanical and thermal hypersensitivities under inflammatory and neuropathic conditions (Keeble et al., 2009; Fidanboylu et al., 2011). In addition, ROS generation has been shown to be involved in itch-related response induced by intradermal injection of leukotriene B₄ (Fernandes et al., 2013). ROS may be associated with a variety of pathologic cutaneous sensations. Thus, we examined whether oxidative stress is responsible for peripheral postischemic licking.

**Materials and Methods**

**Animals.** Male C57BL/6NCr mice (Japan SLC, Inc., Hamamatsu, Japan) were used. The mice were 6 weeks old at the start of experiments and were housed in a room under controlled temperature (21–23°C), humidity (45–65%), and lighting (lights on from 7:00 AM to 7:00 PM) conditions. Food and water were freely available. Experiments were conducted with the approval of the Animal Care Committee of the University of Toyama and according to the guidelines for investigations of experimental pain in animals published by the International Association for the Study of Pain (Zimmermann, 1983).

**Agents.** N-{Acetyl-1-cystein} (Sigma-Aldrich, St. Louis, MO) and phenyl-N-tert-butyl nitrate (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were dissolved in physiologic saline. The transient receptor potential (TRP) A1 channel blocker HCT-030031 [2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide; Sigma-Aldrich] was dissolved in physiologic saline containing 10% dimethyl sulfoxide and 5% Tween-80. BCTC (N-{4-tert-butylphenyl}-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2H)-carboxamide; Enzo Life Sciences, Plymouth Meeting, PA) was dissolved in regular tap water containing 0.5% carboxymethyl cellulose and 1% Tween-80. Morphine hydrochloride (Sankyo, Tokyo, Japan) was dissolved in physiologic saline; the weight of morphine refers to the salt. Gabapentin (synthesized by H.T. and Y.S.) and pregabalin (Sequioa Research Products Limited, Pangbourne, UK) were dissolved in regular tap water. Capsaicin (Sigma-Aldrich) was dissolved in physiologic saline containing 7.5% dimethyl sulfoxide (for local injection and ocular instillation) or in physiologic saline containing 10% ethanol and 10% Tween-80 (for systemic administration). A solution of 30% H₂O₂ (Wako Pure Chemical Industries, Osaka, Japan) was diluted to 0.1 or 0.3% with physiologic saline.

Morphine has been shown to have antiallodynic effects at subcutaneous doses of 1–5 mg/kg in mice, peaking at 15–30 minutes after injection (Takasaki et al., 2000). Therefore, morphine (1 and 3 mg/kg) was administered subcutaneously 15 minutes before the start of compression ischemia. Gabapentin (100 mg/kg s.c.) and pregabalin (30 mg/kg s.c.) have been shown to have antiallodynic effects in mice, peaking 1–2 hours after injection (Field et al., 2006). Therefore, gabapentin (30 and 100 mg/kg) and pregabalin (10 and 30 mg/kg) were administered subcutaneously 60 minutes before the start of compression ischemia. HCT-030031 has been shown to inhibit hyperalgesia at intraperitoneal doses of 30–300 mg/kg, peaking at 1–2 hours after injection (da Costa et al., 2010). Therefore, HC-030031 (30 and 100 mg/kg) was administered intraperitoneally 60 minutes before the start of compression ischemia or intraplantar H₂O₂ injection. Capsaicin-induced hyperalgesia has been shown to be inhibited by 30-minute pretreatment with BCTC (30 mg/kg p.o.) and Freund’s complete adjuvant–induced hyperalgesia has been shown to be inhibited by 2-hour pretreatment with BCTC (30 mg/kg p.o.) in rats (Pomonis et al., 2003). Therefore, BCTC (30 and 100 mg/kg) was administered orally 60 minutes before the start of compression ischemia or intraplantar capsaicin injection. The doses of N-acetyl-L-cysteine and phenyl-N-tert-butyl nitrate were chosen from our preliminary experiments in which the effects of these agents were examined on licking induced by intraplantar injection of H₂O₂ solution.

**Compression Ischemia of the Hind Paw.** To induce hind-paw ischemia, a glabrous region just proximal to the ankle joint was firmly compressed with a cut rubber band (Oband, no. 16; Kyowa Limited, Osaka, Japan) for 10 minutes (Fig. 1) with the exception of a series of experiments in which the ankle was compressed for 1 or 5 minutes. With the exception of blood-flow assessment (see below), ischemic postischemic responses.

**Assessment of Hind-Paw Blood Flow.** Mice were anesthetized with pentobarbital (70 mg/kg i.p.) and body temperature was maintained at 36.5–37.5°C using a thermostatically-controlled heating pad coupled to a rectal probe (BWT-100; Bio Research Center Co., Ltd., Nagoya, Japan). A laser Doppler flowmeter (ALF21; Advance Co., Ltd., Tokyo, Japan) was used to assess blood flow in the plantar skin of the hind paw. The probe (Type N, 0.5-mm diameter) of the laser Doppler flowmeter was held 1 mm from the plantar surface to avoid mechanical and thermal effects on blood flow. Flux signals were low-pass filtered with a time constant of 3 seconds to reduce movement artifacts.

**Postischemic Licking.** Mice were placed individually in the observation chambers (8 × 10 × 20 cm) with an acrylic transparent floor. After a 30-minute acclimation period, the mice underwent compression ischemia for 10 minutes as described above. Immediately after cutting the rubber tourniquet, the mice were placed back into their chambers and their behaviors were videotaped with no one present. The time spent licking the treated hind paw (see Fig. 1) was measured with a stopwatch during video playback. The mice shook the treated hindlimb following compression-reperfusion, which subsided after a few minutes; this behavior was not quantified in this study.

![Rubber Tourniquet](https://via.placeholder.com/150)

**Fig. 1.** Changes in plantar skin blood flow during ankle compression and after reperfusion. In four mice, the ankle was firmly compressed with a rubber band for 10 minutes, and then the rubber tourniquet was cut for reperfusion. Four control mice did not undergo ankle compression. Plantar skin blood flow was measured with a laser Doppler flowmeter. Data are presented as mean ± S.E.M. The upper diagram illustrates a rubber tourniquet and a region (gray in color) licking of which was measured as postischemic responses.
Agent-Induced Licking. Mice were individually placed in the observation chamber for at least 30 minutes to adapt to the environment. Solutions of capsaicin (0.1 μg) and H2O2 were injected into the plantar region of the unilateral hind paw at a volume of 20 μl. The mice were immediately put back into their chamber and their behaviors were videotaped with no one present. The time spent licking the treated paw was measured with a stopwatch during video playback (Sasaki et al., 2013).

Neonatal Capsaicin Treatment. Capsaicin (50 mg/kg) or vehicle was injected subcutaneously twice on days 2 and 5 after birth (Nakano et al., 2008). To verify sensory C-fiber neuron depletion, one drop (10 μl) of 0.1% capsaicin or vehicle was applied to both eyes, and the number of eye wiping with the forelimbs within 30 seconds was counted (Nakano et al., 2008).

Data Analysis. Data are presented as mean ± S.E.M. For licking behavior, statistical differences between two groups were analyzed with Student’s t test or Mann-Whitney rank sum test. Statistical differences among three or more groups were analyzed with one-way analysis of variance (ANOVA) or Kruskal-Wallis one-way ANOVA on ranks (for data without normal distribution or equal variance) followed by post hoc Dunnett’s test. For licking behavior, statistical differences were analyzed with two-way ANOVA followed by post hoc Tukey’s test. A P value of less than 0.05 was considered significant. Statistical analyses were performed using SigmaPlot graphing and statistical software (version 11.2, Systat Software, Inc., San Jose, CA).

Results

Changes in Plantar Skin Blood Flow during Ankle Compression and after Reperfusion. Tight compression of the ankle with a rubber tourniquet produced prompt (<1 minute) and almost complete occlusion of blood flow to the hind paw (Fig. 1). The loss of blood flow persisted during the 10-minute compression (Fig. 1). After cutting the tourniquet, blood flow was promptly increased up to >200% of the precompression value within 1 minute (Fig. 1). This reactive hyperemia gradually decreased and almost subsided at 10 minutes after tourniquet cutting. There were no significant changes in hind-paw blood flow during the 25-minute period in control mice that did not undergo ankle compression (Fig. 1).

Postischemic Licking. When untreated control mice were returned to the observation chamber, they demonstrated active exploratory behavior and increased locomotor activity for about 10 minutes, with rare licking of the hind paws (Fig. 2A). In contrast, mice demonstrated marked licking of the treated hind paw during the first 10-minute period following I-R (Fig. 2A). The cumulative time of hind-paw licking was significantly (Mann-Whitney rank sum test; P = 0.004) increased during the first 10-minute period compared with that in control animals (Fig. 2B). During the 10- to 30-minute period after I-R, licking of the hind paws was observed mainly as a series of grooming behaviors, and the time spent hind-paw licking was similar between the I-R and control groups (Fig. 2, A and B). These results suggest that hind-paw licking during the first 10-minute period was primarily attributable to I-R. We next investigated the effects of duration of ischemia (1, 5, and 10 minutes) on the I-R–induced hind-paw licking during the first 10-minute period. The cumulative time of hind-paw licking was significantly (one-way ANOVA; F3,22 = 9.753, P < 0.001) increased after compression ischemia in an ischemic duration–dependent manner (Fig. 2C). Since the time of hind-paw licking was longest after 10-minute ischemia, we investigated in the subsequent experiments the pharmacologic characteristics and mechanisms of postischemic licking during the first 10-minute period after 10-minute I-R.

Effects of Morphine, Gabapentin, and Pregabalin on Postischemic Licking. Hind-paw licking following I-R was dose-dependently and significantly (Kruskal-Wallis one-way ANOVA on ranks; P = 0.001) inhibited by subcutaneous injections of morphine (1 and 3 mg/kg) (Fig. 3A). The licking was almost completely inhibited at a dose of 3 mg/kg (Fig. 3A). In contrast, neither gabapentin (30 and 100 mg/kg s.c.) nor pregabalin (10 and 30 mg/kg s.c.) inhibited postischemic licking (Fig. 3, B and C).

Effects of N-Acetyl-L-Cysteine and HC-030031 on Postischemic Licking. The antioxidant N-acetyl-L-cysteine has been used as a tool for investigating the role of ROS in numerous biologic and pathologic processes (Zafarullah et al., 2003). To determine whether reperfusion-induced oxidative stress is involved in postischemic licking, we investigated the effect of N-acetyl-L-cysteine on postischemic licking. N-Acetyl-L-cysteine (100 and 200 mg/kg i.p.) dose-dependently and significantly (Kruskal-Wallis one-way ANOVA on ranks;
P<0.002) inhibited postischemic licking, which was almost completely inhibited at 200 mg/kg (Fig. 4A).

HC-030031 is a potent and selective TRPA1 channel blocker and has been used as a tool for investigating the role of TRPA1 channels in both in vitro and in vivo experiments (McNamara et al., 2007; Fernandes et al., 2013). To determine whether TRPA1 channels are involved in postischemic licking, we investigated the effect of HC-030031. HC-030031 (30 and 100 mg/kg i.p.) dose-dependently and significantly (one-way ANOVA; \(F_{2,21} = 6.234, P = 0.007\)) inhibited postischemic licking (Fig. 4B).

**H\(_2\)O\(_2\)-Induced Hind-Paw Licking and Its Suppression by N-Acetyl-l-Cysteine and HC-030031.** Intraplantar injections of 0.1 and 0.3% H\(_2\)O\(_2\) concentration dependently and significantly (Kruskal-Wallis one-way ANOVA on ranks; \(P < 0.001\)) elicited hind-paw licking (Fig. 5A). We observed that licking peaked within 1 minute after 0.1% H\(_2\)O\(_2\) injection and then rapidly subsided. Licking after 0.3% H\(_2\)O\(_2\) injection persisted for a longer period and decreased more slowly. Licking induced by 0.3% H\(_2\)O\(_2\) was significantly (Student’s \(t\) test; \(P = 0.009\)) inhibited by pretreatment with N-acetyl-l-cysteine (200 mg/kg i.p.) (Fig. 5B). H\(_2\)O\(_2\)-induced licking was also significantly (Student’s \(t\) test; \(P = 0.014\)) inhibited by pretreatment with HC-030031 (100 mg/kg i.p.) (Fig. 5C).

**Effects of Local Injection of Phenyl-N-tert-Butylnitrone on Licking Induced by H\(_2\)O\(_2\) Injection and Ischemia-Reperfusion.** We examined the effects of intraplantar injection of another antioxidant, phenyl-N-tert-butylnitrone (Das et al., 2012), on licking induced by H\(_2\)O\(_2\) injection and ischemia-reperfusion to confirm the role of ROS production in the paw distal to the compression site. Intraplantar injections of phenyl-N-tert-butylnitrone (10 and 100 mg/site) together with 0.3% H\(_2\)O\(_2\) dose dependently and significantly (one-way ANOVA; \(F_{2,18} = 11.661, P < 0.001\)) inhibited H\(_2\)O\(_2\)-induced licking, with a significant inhibition (Dunnett’s test; \(P < 0.05\)) observed at a dose of 100 mg/site (Fig. 6A). An intraplantar injection of phenyl-N-tert-butylnitrone (100 mg/site) significantly (Student’s \(t\) test; \(P = 0.006\)) inhibited postischemic licking also (Fig. 6B).

**Effects of TRPV1 Channel Blocker on Capsaicin-Induced and Postischemic Licking.** An intraplantar injection of capsaicin (0.1 \(\mu\)g) elicited hind-paw licking; the effect peaked within 1 minute and then subsided in a few minutes (data not shown). Thus, the cumulative time spent licking was measured for 3 minutes after injection. The selective TRPV1 channel blocker BCTC (Pomonis et al., 2003) dose-dependently and significantly (one-way ANOVA; \(F_{2,15} = 8.548, P = 0.003\)) inhibited capsaicin-induced licking at oral doses of 30 and 100 mg/kg (Fig. 7A). In contrast, there was an increased tendency for postischemic licking after BCTC administration at the same doses but this apparent difference...
was not statistically significant (one-way ANOVA; $F_{2,15} = 1.585$, $P = 0.237$) (Fig. 7B).

**Effect of Neonatal Capsaicin Treatment on Postischemic Licking.** Mice underwent neonatal capsaicin treatment to deplete sensory C-fiber neurons expressing TRPV1 channels. A wiping test was performed to confirm the effectiveness of the treatment. Neonatal capsaicin treatment significantly reduced capsaicin-induced eye wiping (Fig. 8A); two-way ANOVA revealed a significant main effect of neonatal capsaicin treatment ($F_{1,12} = 9.437$, $P = 0.01$) and significant interaction between neonatal capsaicin treatment and eye stimuli ($F_{1,12} = 5.11$, $P = 0.043$). However, neonatal capsaicin treatment did not significantly affect postischemic licking (Fig. 8B).

**Discussion**

In human experiments, peripheral postischemic dysesthesias begin approximately 1 minute after release of cuff compression of 5–30 minutes duration and persist for 5–10 minutes (Merrington and Nathan, 1949; Suarez-Roca et al., 2003). Following I-R of the leg, we generally experience spontaneous dysesthesias such as tingling and pricking, and in severe cases tactile stimulation of the toes can elicit pain. In the present study, we found that reperfusion following a 10-minute arrest of blood flow to the hind paw induced licking of the same hind paw for approximately 10 minutes. Because mice could freely move on their extremities, the hind-paw licking might be attributable to spontaneous and touch-evoked dysesthesias; thus, the behavior is a potential indication of acute postischemic dysesthesias in mice.

After release of compression ischemia, blood flow was promptly increased up to >200% of the precompression value within 1 minute and gradually decreased for about 10 minutes. Re-establishment of blood flow in the ischemic tissue restores the supplies of oxygen and glucose that are indispensable for cell survival. However, mitochondria and oxidative stress–promoting enzymes, such as nicotinamide adenine dinucleotide phosphate oxidases also use oxygen, and activation of these generator systems leads to increased ROS generation (Zweier and Talukder, 2006). ROS oxidize proteins, lipids, and intracellular ions (Moran et al., 2001). In the present study, intraplantar injection of H$_2$O$_2$ elicited hind-paw licking, and this behavior was significantly inhibited by intraperitoneal administration of the antioxidant N-acetyl-l-cysteine. Likewise, postischemic licking was inhibited by N-acetyl-l-cysteine. In addition, an intraplantar injection of the antioxidant phenyl-N-tert-butylnitrone inhibited postischemic licking at a dose that suppressed H$_2$O$_2$-induced licking. These results, taken together, suggest that oxidative stress induced by I-R is involved in postischemic licking and that the paw distal to the compression site is a causal tissue.

In the present study, the selective TRPA1 channel blocker HC-030031 inhibited postischemic licking. The selective TRPV1 channel blocker BCTC did not inhibit postischemic licking at a dose that significantly suppressed licking induced by capsaicin, a selective TRPV1 channel agonist. These results suggest that activation of TRPA1, but not TRPV1, plays a key role in the development of postischemic licking. Additionally, oxidative stress can be increased by I-R, and this oxidative stress induces postischemic licking. Moreover, oxidative stress-induced postischemic licking can be inhibited by intraperitoneal administration of N-acetyl-l-cysteine and phenyl-N-tert-butylnitrone. These results suggest that oxidative stress is involved in postischemic licking.
role in postischemic licking. Several lines of evidence indicate that TRPA1 channels are a major oxidation sensor. Various oxidants, such as tert-butyl hydroperoxide, potassium superoxide, H$_2$O$_2$, and 4-hydroxynonenal, directly activate TRPA1 channels (Andersson et al., 2008; Bessac et al., 2008; Sawada et al., 2008). Oxidation of intracellular cysteine residues is critical for TRPA1 channel activation. Six cysteine residues located in the amino-terminal segment have been identified as potential sites for oxidation and activation of TRPA1 channel (Takahashi and Mori, 2011). Cysteine-reducing agents almost completely inhibit TRPA1-mediated Ca$^{2+}$ influx elicited by H$_2$O$_2$ (Sawada et al., 2008). In contrast, cysteine-oxidizing agents elicit TRPA1-mediated Ca$^{2+}$ influx (Sawada et al., 2008). H$_2$O$_2$ is known to oxidize cysteine residues in proteins to form either cysteine sulfenic acids or disulfides (Poole et al., 2004). In the present study, H$_2$O$_2$-induced paw licking was inhibited by HC-030031 as well as N-acetyl-L-cysteine, indicating that H$_2$O$_2$ elicits licking via the activation of TRPA1 channel. Our findings suggest that reperfusion-induced oxidative stress causes postischemic licking via TRPA1 channel activation. On the other hand, TRPV1 channels are activated by capsaicin, noxious heat, and low pH (Caterina et al., 2000) but not oxidants, such as H$_2$O$_2$ and 4-hydroxynonenal (Andersson et al., 2008; Bessac et al., 2008). TRPA1 channels are expressed in myelinated A-fiber as well as unmyelinated C-fiber sensory neurons (Kobayashi et al., 2005; Kwan et al., 2009). Direct administration of H$_2$O$_2$ to dorsal root ganglion neurons elicits TRPA1-mediated Ca$^{2+}$ influx (Andersson et al., 2008; Sawada et al., 2008). Taken together, these findings suggest that ROS directly activates TRPA1 channels in primary sensory neurons to elicit postischemic licking. Because TRPA1 channels are also present in keratinocytes in the skin (Kwan et al., 2009), their activation in keratinocytes may elicit and/or modulate the excitation of primary sensory neurons involved in postischemic dysesthesias.

It has also been proposed that TRPA1 channels are involved in mechanotransduction. In TRPA1-deficient mice, the mechanically evoked firing rate is reduced in both slowly adapting C-fiber nociceptors and Aδ-fiber mechanoreceptors (Kwan et al., 2009). TRPA1 deficiency also reduces the firing rate of slowly adapting Aδ-fibers (Kwan et al., 2009). In contrast to the reduced firing in all subclasses of slowly adapting fibers, TRPA1 deficiency increases mechanically evoked firing in rapidly adapting hair follicle afferents, including D-hair and rapidly adapting Aδ-fibers (Kwan et al., 2009). These findings raise the possibility that subclasses of slowly adapting fibers are involved in the perception of tactile dysesthesia. Thus, we examined the roles of myelinated A-fibers and unmyelinated C-fibers in mediating postischemic licking by degenerating the latter via neonatal capsaicin injections. This treatment is known to cause permanent degeneration of most C-fibers but does not affect A-fibers (Nakano et al., 2008; Sasaki et al., 2008). We found that postischemic licking was unaffected by neonatal capsaicin treatment, suggesting that this behavior is mediated by A-fibers.

Morphine, a μ-opioid receptor agonist, inhibited postischemic licking. μ-Opioid receptors are expressed in C- and Aδ-fiber, but not Aβ-fiber, sensory neurons (Sasaki et al., 2008; Yamamoto et al., 2008). It has been reported that morphine inhibits the activity of rat dorsal horn neurons evoked by stimulation of C- and Aδ-fibers but not that evoked by stimulation of Aβ-fibers (Jurna and Heinz, 1979). Considering
that ablation of C-fibers by neonatal capsaicin treatment did not affect postschismic lacing, Aδ-fibers are likely responsible for postschismic lacing behavior. In the present study, gabapentin (30 and 100 mg/kg) and pregabalin (10 and 30 mg/kg) did not inhibit postschismic lacing. It has been reported that subcutaneous injections of gabapentin (100 mg/kg) and pregabalin (30 mg/kg) markedly inhibit formalin-induced nociceptive behaviors and nerve ligation–induced mechanothermal allodynia in mice (Field et al., 2006). Gabapentin and pregabalin inhibit C-fiber–evoked responses of rat dorsal horn neurons without affecting Aδ-fiber–evoked responses (Tanabe et al., 2006; You et al., 2009). Thus, the present results that gabapentin and pregabalin did not inhibit postschismic lacing support the hypothesis that Aδ-fibers are responsible for postschismic lacing.

Pricking and tingling are recognized as separate sensations (Ochoa and Torebjörk, 1980; Suarez-Roca et al., 2003). Both are localized to the skin, but pricking is very intense, almost painful, whereas tingling is a less intense sensation with marked rhythmicity (Ochoa and Torebjörk, 1980; Suarez-Roca et al., 2003). Electrophysiologic recordings of human sensory fiber activity following the release of compression ischemia of the arm have suggested that pricking and tingling sensations arise in thinly myelinated Aδ and large myelinated Aβ afferent fibers, respectively (Ochoa and Torebjörk, 1980). Thus, pricking sensations may be more responsible for postschismic lacing than tingling sensations.

Oxidative stress occurs in neuropathic conditions, including diabetic peripheral neuropathy and chemotherapy-induced peripheral neuropathy (Head, 2006), which are accompanied by mechanical allodynia and spontaneous dysesthesias, such as pricking and tingling sensations (Bastyr et al., 2005; Driessen et al., 2012). It has been shown that ROS increases in diabetes and that oxidative stress is involved in diabetic peripheral neuropathy (Singh et al., 2014). ROS production by the antioxidant drug paclitaxel has been demonstrated in cultures of HeLa cells and cortical neuronal cells (Jang et al., 2008; Kim et al., 2008). Paclitaxel also increases the number of atypical (swollen and vacuolated) mitochondria in sensory axons, suggesting mitochondrial injury in peripheral neuropathic conditions (Flatters and Bennett, 2006; Jin et al., 2008). The pivotal role of ROS in neuronal injuries has been further demonstrated by the beneficial impact of N-acetyl-l-cysteine therapy in the treatment of diabetic neuropathy in rats (Kamboj et al., 2010) and neuropathy induced by oxaliplatin, a chemotherapeutic agent, in humans (Lin et al., 2006). Peripheral blood flow is decreased in conditions of diabetic neuropathy (Maxfield et al., 1997) and neuropathy induced by paclitaxel and oxaliplatin (Gauchan et al., 2009). Taken together, these findings suggest that sudden increase in peripheral blood flow in these pathologic conditions causes dysesthesia and allodynia through activation of the ROS-TRPA1 pathway.

In summary, our results demonstrate that reperfusion after brief hind-paw ischemia induces hind-paw lacing following by transient reactive hyperemia in mice. Moreover, our findings suggest that reperfusion-induced oxidative stress activates TRPA1 channels to provoke peripheral postschismic lacing, which is mediated by myelinated (probably Aδ-type) afferent fibers. Targeting oxidative stress with antioxidants or inhibiting TRPA1 channels using selective TRPA1 channel blockers may be novel and effective therapeutic options for peripheral ischemia–associated allodynia and dysesthesias.

Authorship Contributions

Participated in research design: Sasaki, Andoh, Taniguchi, Kuraishi.

Conducted experiments: Sasaki, Mizoguchi, Kagaya, Shiro, Sakai, Kino.

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References


Peripheral Postischemic Dysesthesia in Mice

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