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Sensory nerves contribute to cutaneous vasodilator response to cathodal stimulation in healthy rats

Stéphanie Gohin [a], Johanna Decorps [b,c], Dominique Sigaudo-Roussel [b,c], Bérengère Fromy [b,c,*]

[a] Department of Comparative and Biomedical Sciences, Royal Veterinary College, London NW1 0TU, United Kingdom
[b] Laboratory of Tissue Biology and Therapeutic Engineering UMR5305 University of Lyon 1 and Centre National de la Recherche Scientifique (CNRS) IBCP, 7 passage du vercors 69367 Lyon cedex France
[c] University of Lyon 1, UMR 5305, Lyon, France

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ABSTRACT

Cutaneous current-induced vasodilation (CIV) in response to galvanic current application is an integrative model of neurovascular interaction that relies on capsaicin-sensitive fiber activation. The upstream and downstream mechanisms related to the activation of the capsaicin-sensitive fibers involved in CIV are not elucidated. In particular, the activation of cutaneous transient receptor potential vanilloid type-1 (TRPV1) channels and/or acid-sensing ion channels (ASIC) (activators mechanisms) and the release of calcitonin gene-related peptide (CGRP) and substance P (SP) (effector mechanisms) have been tested. To assess cathodal CIV, we measured cutaneous blood flow using laser Doppler flowmetry for 20 min following cathodal current application (240 s, 100 μA) on the skin of the thigh in anesthetized healthy rats for 20 min. CIV was studied in rats treated with capsazepine and amiloride to inhibit TRPV1 and ASIC channels, respectively; CGRP8-37 and SR140333 to antagonize CGRP and neurokinin-1 (NK1) receptors, respectively; compared to their respective controls. Cathodal CIV was attenuated by capsazepine ($12 \pm 2\%$ vs $54 \pm 6\%$, $P < 0.001$), amiloride ($19 \pm 8\%$ vs $61 \pm 6\%$, $P < 0.01$), CGRP8-37 ($15 \pm 6\%$ vs $61 \pm 6\%$, $P < 0.001$) and SR140333 ($9 \pm 5\%$ vs $54 \pm 6\%$, $P < 0.001$) without changing local acidification. This is the first integrative study performed in healthy rats showing that cutaneous vasodilation in response to cathodal stimulation is initiated by activation of cutaneous TRPV1 and ASIC channels likely through local acidification. The involvement of CGRP and NK1 receptors suggests that cathodal CIV is the result of CGRP and SP released through activated capsaicin-sensitive fibers. Therefore cathodal CIV could be a valuable method to assess sensory neurovascular function in the skin, which would be particularly relevant to evaluate the presence of small nerve fiber disorders and the effectiveness of treatments.

Introduction

A significant increase in cutaneous blood flow in response to non-noxious galvanic current application is seen in humans (Durand et al., 2002a; Ferrell et al., 2002; Grossmann et al., 1995), as observed following transcutaneous electrical nerve stimulation (Westerman et al., 1987). Small sensory afferents appear to be involved in the vascular response to current application (Berliner, 1997; Hamdy et al., 2001). Accordingly, we previously showed in healthy humans that current-induced vasodilation (CIV) resulted primarily from neural excitation and mainly through activation of capsaicin-sensitive fibers, since it is abolished under local anesthesia and severely impaired after chronic capsaicin desensitization (Durand et al., 2002b). However the question as to the mechanisms involved in stimulating the sensory nerves in response to iontophoretic current application to the skin remains unresolved. Although initially viewed as a source of difficulty in interpreting vascular responses to the iontophoresis of pharmacological agents, this capsaicin-sensitive vascular response to galvanic current application represents an easy integrative model to test the sensory neurovascular interaction in the skin. Because such assessment has the potential to provide information on the presence of neurovascular disorders and the effectiveness of treatment and may even serve as a means for the early detection of disease onset, a better understanding of the underlying mechanisms involved in CIV is thus of major interest. Therefore, the present study was designed to elucidate the upstream and downstream mechanisms related to the activation of the capsaicin-sensitive fibers involved in CIV.

Although iontophoretic current applications are not painful, it is likely that the sensitivity of CIV to local anesthesia and chronic treatment with capsaicin reflects, at least in part, the stimulation of cutaneous C-fiber nociceptors that trigger vasodilation through an axon reflex (Berliner, 1997; Hamdy et al., 2001). Indeed, the axon reflex mechanism activates capsaicin-sensitive fibers by acting directly on cutaneous nociceptors such as transient receptor potential vanilloid type-1 (TRPV1) and/or acid-sensing ion channels (ASIC), which are abundantly expressed in human epidermis and keratinocytes (Inoue et al.,

* Corresponding author at: UMR CNRS 5305; Laboratory of Tissue Biology and Therapeutic Engineering, IBCP, 7 Passage du Vercors, F-69367 Lyon Cedex, France.
E-mail address: berengere.fromy@univ-lyon1.fr (B. Fromy).

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2002; Jiang et al., 2006; Southall et al., 2003; Yamamura et al., 2008). We thus suggested that the activation of the capsaicin-sensitive fibers could result from the excitation of cutaneous TRPV1 and/or ASIC channels in response to the cathodal current stimulation.

A heterogeneous series of physical and chemical agents has been shown to activate and/or potentiate the activity of TRPV1, including protons (pH 6–5) (Bevan and Geppetti, 1994; Caterina et al., 1997; Dhaka et al., 2009; Tominaga et al., 1998; Voets et al., 2004). The variations of pH activate also the ASIC channels (Vick and Askwith, 2015). In addition, it is generally believed that TRPV1 and ASIC channels mediate the greater part of acid-induced nociception in mammals (Devesa et al., 2014; Mitchell et al., 2014; Rocha-Gonzalez et al., 2009; Ugawa et al., 2005). Regarding the upstream mechanism, we thus hypothesized that the current application could change skin pH to activate capsaicin-sensitive fibers by acting directly on cutaneous nociceptors such as transient receptor potential vanilloid type-1 (TRPV1) and/or acid-sensing ion channels (ASIC).

Regarding downstream mechanisms related to the activation of the capsaicin-sensitive fibers, it is well known that activated primary afferent endings commonly release calcitonin gene-related peptide (CGRP) and substance P (SP), two potent vasodilators that are co-localized in subepidermal and epidermal capsaicin-sensitive fibers (Gibbins et al., 1985). Indeed the capsaicin-sensitive sensory system subserves a dual sensory-efferent function whereby initiation of afferent signals and neuropeptide release are coupled at the same nerve endings, as previously reviewed (Szolcsanyi, 2004). In the skin, direct evidence of CGRP and SP release has been obtained using capsaicin, as well as by antidromic electrical nerve stimulation (Kilo et al., 1997; Kress et al., 1999; Schmelz and Petersen, 2001).

The present study examined the hypothesis that cathodal stimulation activates capsaicin-sensitive fibers through local acidification associated with the activation of cutaneous TRPV1 and/or ASIC channels (upstream mechanisms) and CGRP and/or SP release (downstream mechanisms). Cathodal CIV was examined after local blockade of TRPV1, ASIC, CGRP and NK1 receptors in anesthetized healthy rats.

Methods

Animal preparation

Experiments were performed on male Wistar rats weighing 200–400 g aged from 7 to 10 weeks. Before the experiments, animals had free access to standard laboratory food and water and were housed in a regulated environment with a constant ambient temperature of 24 °C. Procedures for the maintenance and use of animals were carried out in accordance with the principles of French legislation and the experiments were approved (no. 0224) by the ethics committee for animal experimentation of the University of Lyon, France.

The hair was removed from the skin of the thighs with a depilatory lotion to present hairless areas for cutaneous blood flow measurements and local current application. This depilation was performed two days before the experiments to avoid skin irritation at the time of the studies.

For the experiments, animals were anesthetized with Nesdonal (50 mg/kg body weight, i.p.). The rats were then placed in the prone position in an incubator (MP4SI, Mediprema, Chambray les Tours, France) warmed to maintain a stable cutaneous temperature throughout the experiment (35.5 ± 0.1 °C) measured using a thermocouple (Physitemp BAT-12, Physitemp Instruments Inc., Clifton, USA) placed near the site selected for current application. Systolic arterial blood pressure was measured prior to current application and at the end of the experiment using a pressure transducer placed on the tail (XBP-1000, Kent Scientific, Torrington, USA). Only rats with stable systolic arterial blood pressure (difference between basal and final values less than 20 mm Hg) were included in the study to ensure hemodynamic stability.

Assessment of cathodal CIV

The cutaneous blood flow response to cathodal current application (240 s, 100 μA), through deionized water, was measured from the surface of the skin of the thigh of the anesthetized rats using an iontophoretic device that was adapted to hold a laser Doppler probe (PF 4001 Master, Periflux, Perimed, Sweden) at the center of the stimulated site. The laser Doppler signals were expressed in arbitrary units (a.u.). For the purpose of the study, all pharmacological agents were administered into the skin at the site of the current stimulation using either topical treatment or subcutaneous (s.c.) injections (0.1 ml). Animals were sacrificed at the end of the experiment by an overdose of the anesthetic agent.

Protocol 1: nerve fiber involvement in cathodal CIV

To examine the involvement of nerve fibers, CIV was assessed in rats treated with a local application of a topical anesthetic cream (2.5%–2.5% lidocaine-prilocaine cream, $n = 10$) and in control rats ($n = 10$) receiving a local application of a placebo cream (paraffin/vaseline mixture). Anesthetic and placebo creams were applied topically to the skin 30 min prior to the start of the experiment. As previously used in humans (Durand et al., 2002b), this exposure time to lidocaine–prilocaine cream was chosen to block neural activity and avoid its vasodilator effect observed with prolonged application (Bjerring et al., 1989).

Protocol 2: TRPV1 channel involvement in cathodal CIV

To examine the involvement of TRPV1 channels, CIV was assessed in rats treated with a TRPV1 antagonist (capsazepine, 0.024 mmol/ml, s.c., $n = 10$) as previously used in rats (Wang et al., 2008). The control rats received the same volume of the corresponding vehicle (DMSO 45%, s.c., $n = 10$) than the treated rats. Capsazepine and DMSO 45% were injected 20 min prior to the start of the experiment.

Protocol 3: ASIC channel involvement in cathodal CIV

To examine the involvement of ASIC, CIV was assessed in rats treated with a non-specific ASIC inhibitor (amiloride, 0.5 mmol/ml, s.c., $n = 10$) as previously used in rats (Dube et al., 2005; White et al., 2002) and in control rats that received the same volume of the corresponding vehicle (saline, s.c., $n = 10$) than the treated rats. In addition to its ability to inhibit ASIC channels, amiloride is a sodium proton exchange (NHE) inhibitor (Masereel et al., 2003). To control for that property of amiloride, we also tested the effect on CIV of the potent NHE inhibitor cariporide (HOE 642, 0.002 mmol/ml, s.c., $n = 10$), as previously used in rats (Aye et al., 1997). Amiloride, cariporide and saline were injected 30 min prior to the start of the experiment.

Protocol 4: skin surface pH

Conventional surface pH measurements were performed using a flat glass surface electrode (Metrohm, France) with a pH meter (pH Meter 827; Metrohm, France) placed on the skin. In untreated rats, skin surface pH was measured for 20 min with ($n = 10$) and without ($n = 10$) cathodal stimulation (240 s, 100 μA). Skin surface pH was also measured in response to cathodal stimulation (240 s, 100 μA) in rats treated with capsazepine (0.024 mmol/ml, s.c., $n = 10$) and their respective control rats (DMSO 45%, s.c., $n = 10$) and in rats treated with amiloride (0.5 mmol/ml, s.c., $n = 10$) and their respective control rats (saline, s.c., $n = 10$).