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The involvement of TRPA1 channel activation in the inflammatory response evoked by topical application of cinnamaldehyde to mice

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ABSTRACT

Aims: In the present work, we characterize the inflammatory process induced by the topical application of cinnamaldehyde on the skin of mice and verify the participation of transient receptor potential A1 TRPA1 receptors in this process.
Main methods: We measured mouse ear edema and sensitization/desensitization after topical application of cinnamaldehyde or/and capsaicin. We also quantified cellular infiltration through myeloperoxidase (MPO) activity and histological and immunohistochemical analyses and evaluated the expression of TRPV1 and TRPA1 by western blot.
Key findings: Cinnamaldehyde induced ear edema in mice (1–6 μg/ear) with a maximum effect of 4 μg/ear. Cinnamaldehyde promoted leukocyte infiltration as detected by increasing MPO activity and confirmed by histological analyses. The edema and cellular infiltration evoked by the application of 4 μg/ear of cinnamaldehyde were prevented by topical application of ruthenium red, a non-selective TRP antagonist as well as camphor and HC030031, two TRPA1 receptor antagonists. Cinnamaldehyde-induced edema, but not cellular infiltration, was prevented by topical application of the tachykinin NK1 antagonist, aprepitant, indicating a neuropeptide release phenomenon in this process. Additionally, we observed that repeated topical applications of cinnamaldehyde did not induce changes in sensitization or desensitization with respect to the edema response. Interestingly, repeated treatment with the TRPV1 agonist, capsaicin, abrogated it edematogenic response, confirming the desensitization process and partially decreasing the cinnamaldehyde-induced edema, suggesting the involvement of capsaicin-sensitive fibers.
Significance: Our data demonstrate that the topical application of cinnamaldehyde produces an inflammatory response that is dependent on TRPA1 receptor stimulation.

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Introduction

Various natural compounds can induce contact irritant dermatitis upon skin exposure. Commonly used products such as cosmetics may contain these ingredients. Animal and human studies have demonstrated the irritant properties of several compounds, such as cinnamaldehyde, which is found in cinnamon oil and used in body lotions, hair sprays, shampoos and face creams (Cocchiara et al., 2005). Cinnamaldehyde is considered a moderately potent sensitizing hapten that is capable of penetrating the skin, and the use of up to 3% cinnamaldehyde can induce dermal irritation in human volunteers. In spite of this, 10% cinnamaldehyde can be found in some cosmetic products. However, the mechanism by which this irritant compound produces skin reactions is still unclear (Johansen et al., 1996; Bickers et al., 2005; Thorne et al., 1991).

Other natural skin irritants, such as allyl isothiocyanate from mustard oil and capsaicin from red pepper, produce dermatitis by stimulating members of the transient receptor potential (TRP) family of ion channels. TRP channels are widely expressed in mammalian tissues and play diverse roles in sensorial detection. These channels involve six related protein subfamilies that comprise the TRPV1 (vanilloid subfamily) and the TRPA1 (ankyrin subfamily) receptors,

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which are gated by diverse stimuli, such as chemical and thermal activation (Caterina et al., 1997; Story et al., 2003; Bandell et al., 2004; for review see: Calixto et al., 2005).

TRPV1, a heat-activated channel (≥43 °C), is stimulated by a broad array of natural, synthetic and endogenous substances, such as capsaicin, resiniferatoxin and anandamide. It has been hypothesized that TRPA1 could be activated by mechanical and cold stimuli, but this is still under debate (Corey et al., 2004; Bandell et al., 2004; Kwan et al., 2006; Jordt et al., 2004; Caspani and Heppenstall, 2008). Also, TRPA1 is chemically activated by a wide range of natural and synthetic compounds, including endogenous ligands (4-hydroxynonenal), and several irritant substances (allyl isothiocyanate and cinnamaldehyde) (Trevisani et al., 2007; Bautista et al., 2005; for review see: Calixto et al., 2005; Zurborg et al., 2007). Allyl isothiocyanate and capsaicin have been historically used as chemical irritants in models of ear edema in rodents. Their application results in characteristic neurogenic inflammation, which is observed as increased blood flow, vascular permeability, leukocyte infiltration and release of neuropeptides into the rodent skin, a phenomenon that is also observed in some cases of dermatitis (Jancsó et al., 1977; Gamse et al., 1980; Gábor and Ra'zga, 1992; Inoue et al., 1997; Huang et al., 2003).

Cinnamaldehyde may induce ear edema in mice (Thorne et al., 1991), however, the effect of cinnamaldehyde on other inflammatory parameters, such as leukocyte infiltration, is unclear. Despite knowing that cinnamaldehyde acts as an agonist of TRPA1, the role of TRPA1 in skin irritation produced by cinnamaldehyde is still unknown. Therefore, the aim of this study was to better characterize the inflammatory process induced by cinnamaldehyde in the skin and to verify TRPA1's participation in this process.

Materials and methods

Animals

Experiments were performed on adult male Swiss mice (weight 25–35 g) bred in our animal house. The animals were kept in a temperature-controlled room (22 ± 2 °C) under a 12-h light–dark cycle. Food and water were freely available. The animals (4 to 6 per group) were acclimatized to the laboratory for at least 24 h before testing and were used only once. All protocols are in accordance with the US guidelines for the care and use of laboratory animals (Zimmermann, 1983) and all procedures were approved by our Institutional Ethics Committee (process numbers 67/2010). The number of animals used was the minimum necessary to demonstrate consistent effects of the drug treatments.

Drugs and reagents

The following substances were used: allyl isothiocyanate, capsaicin, cinnamaldehyde, ammoniated ruthenium oxychloride (ruthenium red, RR), 4′-chloro-3-methoxycinnamanilide (SB366791), ethylene diamine tetraacetic acid (EDTA); 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7 H-purin-7-yl)-N-(4 isopropylphenyl) acetamide (HC030031), formaldehyde, 3,3,5,5-tetramethylbenzidine (TMB), p-nitrofenil-2-acetamida-β-D-glicopiranoside (NAG) and hexa decyltrimethylammonium bromide (HTAB). All substances were purchased from Sigma (St. Louis, MO, USA). Hematoxylin-eosin was purchased from MERCK (Whithouse Station, New Jersey, USA). Sodium acetate, acetone, acetic acid, ethanol, and camphor were purchased from Vetec (Rio de Janeiro, Brazil). Halothane was purchased from Cristália (São Paulo, Brazil) and sodium citrate was purchased from Isofar (Rio de Janeiro, Brazil).

Aprepitant was extracted from commercially available capsules (Emend®, Merck, USA), and its identity and purity were confirmed by nuclear resonance methods and were >98%.

Ear edema measurement

Edema was expressed as an increase in ear thickness due to the chemical irritant challenge. Ear thickness was measured before and after induction of the inflammatory response using a digital micrometer (Starret Series 734) in animals anesthetized with halothane. The micrometer was applied near the tip of the ear just distal to the cartilaginous ridges. The thickness was recorded in μm. To minimize variation, a single investigator performed the measurements throughout each experiment.

Irritant-induced skin inflammation

Vehicle (acetone, 20 μl/ear) or cinnamaldehyde (8, 15, 32 and 45 nmol/ear corresponding to 1, 2, 4 and 6 μg/ear, respectively) was applied to the right ear by topical administration according to the method described previously, with some modifications (Thorne et al., 1991; Gábor and Ra'zga, 1992). Another group of animals was given topical application of allyl isothiocyanate (10, 20, 40 and 60 nmol/ear corresponding to 1, 2, 4 and 6 μg/ear, respectively) or capsaicin (328, 655 and 983 nmol/ear corresponding to 100, 200 and 300 μg/ear, respectively) on their right ear (Gábor and Ra'zga, 1992; Inoue et al., 1997). The ear thickness was measured before and from 15 min to 8 h after topical application.

Separate groups of animals were topically pretreated with the non-selective TRP antagonist, RR (30–300 nmol/ear), the TRPA1 antagonists, camphor (30–1000 nmol/ear) or HC030031 (30–300 nmol/ear), the selective TRPV1 antagonist, SB366791 (10–100 nmol/ear) or the tachykinin receptor antagonist, aprepitant (30–300 nmol/ear). All compounds were diluted in acetone.

The effect of repeated topical application of capsaicin or cinnamaldehyde

We investigated whether repeated ear exposure to cinnamaldehyde or capsaicin could induce the sensitization/desensitization process to its edematogenic effect. Briefly, mouse ears were topically pretreated on days 1, 3 and 7 with acetone (20 μl/ear), capsaicin (200 μg/ear) or cinnamaldehyde (4 μg/ear) in separate groups, as described previously (Inoue et al., 1997). After 8 days, the animals in each group were challenged with cinnamaldehyde (4 μg/ear), capsaicin (200 μg/ear) or acetone (P.A.). The development of edema was assessed, as described before, on days 1, 3, 7 and 8.

TRPA1 and TRPV1 expression

The effect of the pretreatment with acetone, cinnamaldehyde or capsaicin on days 1, 3 and 7, on expression of the TRPA1 and TRPV1 receptors was assessed by western blot analysis. The assay was carried out as described previously (Ferreira et al., 2005) with minor modifications. The ear tissue was obtained on day 8 of the protocol and then homogenized in a lyses buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 2 mM MgCl2, 1 mM EDTA, 1 mM NaF, 10 μg/mL aprotinin, 10 mM β-glycerophosphate, 1 mM phenylmethanesulphonyl fluoride, 1 mM DTT and 2 mM of sodium orthovanadate. After centrifugation (11,000 ×g for 20 min at 4 °C), the supernatant containing the membrane fraction was collected. The protein content was determined by the method of Bradford (1976) using bovine serum albumin as a standard. Amounts of protein (80 μg) were mixed in loading buffer (200 mM Tris, 10% glycerol, 2% SDS, 2.75 mM b-mercaptoethanol and 0.04% bromophenol blue) and boiled for 5 min. Proteins were separated in 12% sodium dodecyl sulfate–polyacrylamide gels (SDS–PAGE) and transferred to polyvinylidene difluoride membranes according to the manufacturer's instructions (Perkin Elmer, USA). The membranes were incubated with Ponceau stain, which served as a loading control (Calvo et al., 2010). Afterwards, the membranes were dried, scanned and quantified with Scion

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