Role of TRPA1 receptors in skin inflammation induced by volatile chemical irritants in mice

Maíra Macedo Norões[c], Larissa Gonzaga Santos[b]; Elaine Cristina Gavioli[a], Vanessa de Paula Soares Rachetti[a], Michel Fleith Otuki[b], Daniela de Almeida Cabrini[b], Arthur da Silveira Prudente[b,f], Janiana Raíza Jentsch Matias Oliveira[b], Muryel de Carvalho Gonçalves[c], Juliano Ferreira[c], Delia Preti[d], Francesco De Logu[e], Romina Nassini[e], Eunice André[b*]

[a]Department of Biophysics and Pharmacology, Federal University of Rio Grande do Norte, Campus Universitário UFRN – Lagoa Nova, Natal, RN, Brazil.

[b]Department of Pharmacology, Federal University of Paraná, Centro Politécnico – Jardim das Américas, Curitiba, PR, Brazil.

[c]Department of Pharmacology, Federal University of Santa Catarina, Campus Universitário – Córrego Grande, Florianópolis, SC, Brazil.

[d]Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Ferrara, Italy.

[e]Department of Health Sciences, Section of Clinical Pharmacology and Oncology, University of Florence, Florence, Italy.

[f]Federal University of Latin American Integration, Avenida Silvio Américo Sasdelli – Bairro Itaipu A, Foz do Iguaçu, PR, Brazil.

Corresponding author

Eunice André

E-mail address: andreeu@hotmail.com

Department of Pharmacology,

Federal University of Paraná, Curitiba, PR, Brazil – 81531-980

Telephone: (55) 413361-1693, Fax: (55) 413266-2042,

Abstract

Contact dermatitis is a very common inflammatory reaction in the skin, causing not only aesthetic problems but also loss functionality at work. The molecular mechanisms of contact dermatitis induced by chemical irritants are still unclear. Considering that transient receptor potential channels (TRP) may induce neurogenic inflammation and the exacerbation of inflammatory responses, here we investigated the role of transient receptor potential channel ankyrin type-1 (TRPA1) in skin inflammation evoked by chemical irritants. Ear oedema and nociceptive responses elicited by the topical application of xylene and toluene were measured in Swiss mice, wild type and TRPA1 knockout (*Trpa1*$^{-/-}$) C57BL/6 mice. Histological analyses were performed in mice subjected to the ear oedema assay. Topical application of xylene and toluene in the mouse ear induced an edematogenic response (0.113±0.008 mm and 0.067±0.011 mm), compared to vehicle (0.008±0.008 mm), assessed by ear thickness measurements and histological analyses. These responses were prevented by topical pretreatment with a selective TRPA1 antagonist, HC-030031 (% inhibition: xylene 36.8±9.4% and toluene 50.7±11.0%), and by the genetic deletion of TRPA1 ((% inhibition: xylene 66.6±16.7% and toluene 75±0%). In addition, the topical application of xylene and toluene to the mouse paw elicited nociceptive responses, which were significantly reduced by oral treatment with HC-030031 ((% of inhibition: 84.9±1.3% and 27.1±8.0%, respectively); nociceptive responses were almost completely abolished in *Trpa1*$^{-/-}$mice. Our data suggest that the activation of TRPA1 could be involved in some of the symptoms of irritant-mediated contact dermatitis, such as oedema, pain and neurogenic inflammation.

Keywords: Transient receptor potential ankyrin 1; inflammatory skin disease; chemical irritants; irritant contact dermatitis;

1. Introduction

Nowadays, due to increasing exposure to chemical agents and environmental pollutants, the number of people developing inflammatory reactions in the skin has increased. Most of these are clinically described as contact dermatitis, which can cause not only aesthetic problems, but also loss functionality at work (Kaplan et al., 2012). Epidemiological studies show that contact dermatitis accounts for 50% of all occupational diseases in the United States, and is the fourth leading cause of work-related disability (Cao and Taylor, 2008; Ngo and Maibach, 2010). The incidence varies according to the risk of exposure of the population to chemical agents, and is higher in chemical industry workers, household workers, painters and hairdressers, among others (Sasseville, 2008).

Both types of contact dermatitis, i.e. irritant-mediated and allergic, are caused by dermal exposure to chemical agents found in detergents, solvents, insecticides, beauty and house cleaning products (Cao and Taylor, 2008). While the allergic reaction results from an immune response triggered in the epidermis and dermis after a previous sensitisation (Cao and Taylor, 2008), irritant-mediated contact dermatitis is caused by chemical agents that directly irritate the skin (Sasseville, 2008; Ngo and Maibach, 2010). Currently, a family of channels known as transient receptor potential channels (TRPs) are considered to be molecular integrators of a large number of noxious chemical stimuli (Boonen et al., 2016). This receptor family has been studied with great interest in several pathologies including skin inflammation (for a review, see Caterina and Pang, 2016). Among these receptors, we highlight the transient receptor potential channels ankyrin type-1 (TRPA1).

TRPA1 channels are activated by pungent chemicals such as horseradish, mustard and cinnamon oils, temperatures less than or equal to 17°C, endogenous inflammatory mediators, as well as two α,β-unsaturated aldehydes (acrolein and crotonaldehyde) generated from the combustion process of cigarette smoking and petrochemical fuels (Bautista et al. 2006; Andrè et al., 2008; Nilius et al., 2012). TRPA1 is expressed in primary sensory fibers and non-neuronal cells such as keratinocytes (Atoyan et al., 2009; Bíró and Kovács, 2009). Some studies have demonstrated that TRPA1 is involved in inflammatory skin symptoms such as erythema, pain and heat hyperalgesia (Silva et al., 2011; Nilius et al., 2012; Brederson et al., 2013). For example, chemicals used in the manufacturing sector, i.e. xylene and formaldehyde, when topically applied to the skin or injected via the intraplantar route, respectively, result in nociception in mice via TRPV1 and TRPA1 activation (McNamara et al., 2007; Sándor et al., 2009; Tian et al. 2009). In addition, it has also been reported that toluene diisocyanate, a hazardous irritant used in paints and foams, causes sensory nerve activation in vitro via TRPA1

gating (Taylor-Clark et al., 2009). However, whether the activation of TRPs, in particular TRPA1, is essential to the development of inflammatory processes, pain and other symptoms observed during irritative contact dermatitis caused by these volatile organic compounds remains unclear. In this context, we investigated the role of TRPA1 in the cutaneous inflammatory responses evoked by chemical irritants such as xylene and toluene after topical application to the skin.

2. Material and methods

2.1 Animals

Experiments were performed on adult male Swiss mice (25-30 g) and wild type (*Trpa1*$^{+/+}$) or *Trpa1*-deficient (*Trpa1*$^{-/-}$B6;129P-Trpa1tm1Kykw/J) mice generated in heterozygous animals on a C57BL/6 background (Jackson Laboratories, Bar Harbor, Maine, USA). Animals were maintained in standard housing conditions (12h light/dark cycle, lights on at 6 a.m., food and water *ad libitum* in a climatically controlled environment). The experimental procedures were performed according to the ARRIVE guidelines for reporting experiments involving animals (Kilkenny et al., 2010) and with the standards set in the directives for animal care in Brazilian law n°11.794/2008. All protocols employed have been approved by the Ethics Committee of the Federal University of Rio Grande do Norte (Protocol n°: 023/2010) and Federal University of Paraná (Protocol n°: 698), except for the experiments with *Trpa1*$^{+/+}$ or *Trpa1*$^{-/-}$mice. These were conducted at the University of Florence (research permit #204/2012-B) in conformity with the European Communities Council (ECC) guidelines for animal care procedures and Italian legislative application (DL 116/92) of the ECC directive (86/609/EEC).

2.2 Drugs and reagents

The TRPA1 antagonist 1,2,3,6-Tetrahydro-1,3-dimethyl-N-[4-(1-methylethyl)phenyl]-2,6-dioxo-7H-purine-7-acetamide, 2-(1,3-Dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-N-(4-isopropylphenyl)acetamide (HC-030031) was synthesised and purified as previously described (Andrè et al., 2008) and the structure of the compound was assigned by H-NMR and mass spectroscopy (final purity >98%) determined by RP-HPLC. Toluene and xylene were purchased from Sigma (St. Louis, MO, USA). All drugs used topically were dissolved in acetone immediately prior to use. HC-030031

was prepared in 0.9% NaCl containing 5% dimethylsulphoxide plus 5% Tween 80 for intragastric administration. The solutions were diluted on the day of the experiment immediately before use.

2.3 Volatile chemical irritant-induced ear oedema

Oedema induced by the topical application of chemical irritants was measured as an increase in ear thickness. The chemical irritants were pipetted directly onto the ears of the mice. Ear thickness was assessed with an electronic digital micrometer (Starrett Series 734) applied near the tip of the ear distal to the cartilaginous ridges; the difference in thickness between the basal measurement and after challenge was calculated (Silva et al., 2011). To minimise variation, a single investigator performed the measurements and the values were recorded in mm. Ear thickness was measured before and until 120 min after the topical application of xylene (100%/20 µl/ear), toluene (100%/20 µl/ear) or their vehicle (acetone, 20 µl/ear) onto the right ear.

2.4 Involvement of the TRPA1 receptor in the edematogenic response induced by volatile chemical irritants

Separate groups of animals were topically pretreated with the TRPA1 selective antagonist HC-030031 (300 nmol/20 µl/ear) (Silva et al., 2011) or its vehicle (acetone, 20 µl/ear) 15 min before challenge with the organic compounds. Ear thickness was measured before and 30 min after the topical application of xylene (100%/20 µl/ear), toluene (100%/20 µl/ear) or vehicle (acetone, 20 µl/ear) to the right ear. In another set of experiments, oedema induced by the topical application of organic compounds in wild type and *Trpa1*$^{-/-}$mice was evaluated until 120 min after exposure.

2.5 Histology

Histological changes were evaluated in ear samples from mice pretreated with the topical TRPA1 antagonist HC-030031 (300 nmol/20 µl/ear) or vehicle in *Trpa1* wild type and *Trpa1*$^{-/-}$mice, challenged with the organic compounds. Thirty min after the induction of oedema by xylene or toluene, mice were euthanised with thiopental (100 mg/kg, intraperitoneal) and ear tissues were removed and fixed in alfac solution (16:2:1

mixture of 80% ethanol, 40% formaldehyde and glacial acetic acid). Each sample was embedded in paraffin wax, sectioned at 5 μm and stained with haematoxylin-eosin. A representative area was selected for qualitative light microscopic analysis of the inflammatory cellular response with a 40x objective lens (Recio et al., 2000). These analysis were carried out by using imagej program. To minimise sources of bias, the investigator analysed the specimens blindly.

2.6 Measurement of behavioural nociceptive responses

In this study, we also investigated whether xylene or toluene could induce acute nociception after applying irritant compounds directly to mouse skin. Briefly, animals were firstly individually placed in transparent glass chambers for 1h of acclimation. After the adaptation period, xylene (30, 50, 100%/20 μl/paw) or toluene (30, 50, 100%/20 μl/paw) was topically applied (using a Gilson P100 pipette) onto the plantar surface of the right hind paw. Immediately after the topical application, mice were gently introduced into the chamber for the evaluation of the acute nociceptive response. The amount of time spent licking the exposed hind paw for 5 min after chemical irritant application was recorded with a chronometer and was considered a measure of acute nociceptive behaviour.

2.7 Involvement of the TRPA1 receptor in volatile chemical irritant-induced nociceptive responses

To assess the role of the TRPA1 receptor in the nociceptive response, mice received a single oral dose of a TRPA1 antagonist HC-030031 (100 mg/kg; intragastric) (Eid et al., 2008) or vehicle. Sixty min later, xylene (50%/20 μl) or toluene (50%/20 μl) was applied to the right hind paw and the index of the nociceptive response was evaluated for a period of 5 min. In another set of experiments, xylene (50%/20 μl) or toluene (50%/20 μl) was applied to the right hind paw of wild type and *Trpa1*$^{-/-}$ mice and the nociceptive response was evaluated for a period of 5 min.

2.8 Statistical analysis

Data are reported as the mean ± standard error of the mean (S.E.M.) of at least 4 animals per group. The statistical analysis was assessed by the unpaired two-tailed Student's t-test (to evaluate statistical significance between two groups) or by repeated measures two-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison post hoc test. These evaluations were carried out using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Values of $P \leq 0.05$ were considered to be statistically significant.

3. Results

3.1 Effect of topical of xylene or toluene application on ear oedema

Topical application of xylene (100%/20 µl/ear) and toluene (100%/20 µl/ear) induced ear oedema in Swiss mice. The peak of the edematogenic response was observed at 30 min (0.113 ± 0.008 mm and 0.067 ± 0.011 mm, respectively) when compared to vehicle (0.008 ± 0.008 mm) (Fig. 1A, B).

3.2 Effect of pharmacological blockade and genetic deletion of TRPA1 on ear oedema induced by xylene and toluene

In Swiss mice, ear oedema evoked by xylene (100%/20 µl/ear) and toluene (100%/20 µl/ear) was reduced (36.8 ± 9.4% and 50.7 ± 11.0%, respectively) by pretreatment (local application) with the selective TRPA1 antagonist HC-030031 (300 nmol/20 µl/ear, 15 min prior) (Fig. 2A, B). In addition, in *Trpa1*$^{-/-}$mice, the ear oedema induced by xylene and toluene was strongly reduced (66.6 ± 16.7% and 75 ± 0%, respectively) (calculated on the peak of 30 min) when compared with wild type mice (Fig. 3A, B).

3.3 Histological findings

Cross-sections of mouse ears collected 30 min after being xylene or toluene challenge showed differences in the thickness of the histological structures, when compared to

vehicle-treated mice (Fig. 4 A,B,D,E). Observations performed with the 40x objective revealed an edematogenic response, mainly between the skin and the cartilage. In contrast, pretreatment with the TRPA1 antagonist HC-030031 prevented the ear oedema induced by both chemical irritants (Fig. 4 C,F).

Increased mouse ear thickness was observed in wild type mice after a single topical application of the chemical irritants (Fig. 5-6). However, no histological alterations were observed in *Trpa1*$^{-/-}$mice challenged with xylene (Fig. 5) and toluene (Fig. 6).

3.4 Nociceptive response induced by xylene or toluene application to the mouse paw

The topical application of xylene (50 and 100%/20 µl/paw) or toluene (50 and 100%/20 µl/paw) on the plantar surface of the right hind paw of Swiss mice induced licking reactions in a dose-dependent manner, characteristic of a painful sensation (Fig. 7A, B).

3.5 Effects of pharmacological and genetic blockade of the TRPA1 receptor on xylene or toluene-induced nociceptive responses

Xylene (50%/20 µl/paw) and toluene (50%/20 µl/paw) evoked nociceptive responses were significantly reduced (% of inhibition: 84.9 ± 1.3% and 27.1 ± 8.0%, respectively) by oral pretreatment with the TRPA1 antagonist HC-030031 (100 mg/kg, 60 min prior the chemical application) in Swiss mice (Fig. 8A, B). Furthermore, the nociceptive response elicited by the topical application of xylene (50%/20 µl/ paw; 32.0 ± 5.1 s) and toluene (50%/20 µl/paw; 17.4 ± 2.3 s) was suppressed in mice with genetic deletion of TRPA1 (0.3 ± 0.1 s and 2.4 ± 0.8 s, respectively) as compared to wild type mice (Fig. 9A, B).

4. Discussion

Exposure to noxious chemical compounds may occur throughout multiple routes, including dermal contact. Xylene and toluene are common pollutants in the plastic, chemical and leather industries. Skin contact with chemical substances may result in local diseases such as irritant contact dermatitis, the most common type of occupational

skin disorder, or allergic dermatitis (Sasseville, 2008). The present study shows that the topical application of chemical irritants, i.e. xylene and toluene, induces robust and long-lasting ear oedema in mice.

By focusing on the role played by TRPA1 signalling in the mediation of skin inflammation induced by xylene and toluene, here we show through ear thickness measurements and histological analyses that pharmacological and genetic blockade of TRPA1 significantly inhibited xylene- and toluene-induced ear oedema. Thus, these findings suggest that some volatile irritant compounds, after contact with the skin, promote vascular reactions mediated by TRPA1 receptor signalling. The role of TRPA1 in skin inflammation has been previously reported by our research group (Silva et al., 2011). We showed that the topical administration to the mouse ear of the TRPA1 receptor agonist cinnamaldehyde promoted inflammatory responses with oedema formation, which was inhibited by pre-exposure to the TRPA1 antagonist HC-030031 (Silva et al., 2011). To reinforce the role of TRPA1 in skin disorders, it has been demonstrated that this channel is expressed in many non-neuronal cell types, such as mast cells, keratinocytes (Bíró and Kovács, 2009) and melanocytes (Bellono et al., 2013), and in sensory nerves, where it works as a nociceptive sensor (Atoyan et al., 2009).

It was interesting to note that, besides oedema and erythema, other relevant clinical manifestations mentioned by patients after dermal exposure to chemical irritants are pain sensations and itching at the site of injury (Ngo and Maibach, 2010). We also observed that xylene and toluene contact with the skin induced an acute nociceptive response. In fact, the topical application of xylene and toluene directly to the mouse paw was sufficient to elicit nociceptive responses, as assessed by an increased amount of time spent licking the treated paw. Taken together, the sensory and vascular manifestations observed after the application of xylene and toluene to the ear and paw of mice mimic the main symptoms of irritant contact dermatitis.

In the present study, we also observed that both pretreatment with the TRPA1 antagonist HC-030031 and genetic deletion of the TRPA1 receptor significantly inhibited xylene-and toluene-induced nociceptive responses when topically applied to the mouse paw. A growing body of evidence supports a role for TRPA1 in mediating pain and pruritic responses caused by several exogenous noxious stimuli (Oh et al., 2013; Wilson et al., 2013; Chen and Hackos, 2015). However, Sándor et al. (2009) employed a similar method to that used in this study and reported that xylene-induced nociception is mediated by the TRPV1 receptor signalling. These authors showed that pharmacological and genetic blockade of TRPV1 partially prevented xylene-induced nociceptive responses (Sándor et al., 2009).

It is interesting to note that a close relationship between TRPA1 and TRPV1 has already been reported. Both TRPV1 and TRPA1 are co-expressed in a large subset of sensory nerves and, using transient co-expression of these two receptors in Chinese hamster ovary cells, Staruschenko et al. (2010) demonstrated a direct interaction between TRPV1 and TRPA1, resulting in heterotetramer formation by these two ion channels. Recently, it has also been demonstrated that Ca^{2+} influx through TRPV1 opening may increase the sensitivity of the TRPA1 channel (Hsu and Lee, 2015). Therefore, it is possible that both TRPA1 and TRPV1, working synergistically, can mediate the cutaneous inflammatory response caused by some volatile irritant chemical compounds.

5. Conclusion

In conclusion, we have shown that the activation of TRPA1 could be involved in some of the symptoms of irritant contact dermatitis, such as pain, neurogenic inflammation and possibly itching induced by the topical exposure of some volatile chemical irritants. The present data also support a role for TRPA1 antagonists as innovative drugs for topical pharmacological intervention during skin inflammation. However, other studies are required to further clarify the role of other TRP receptors in inflammatory skin reactions.

Ethical approval and consent to participate

All protocols employed have been approved by the Ethics Committee of the Federal University of Rio Grande do Norte (Protocol n°: 023/2010) and the Federal University of Paraná (Protocol n°: 698), except for the experiments with Trpa1+/+ or Trpa1-/- mice. These were conducted at the University of Florence (research permit #204/2012-B) in conformity with the European Communities Council (ECC) guidelines for animal care procedures and the Italian legislative application (DL 116/92) of the ECC directive (86/609/EEC).

Consent for publication

Not applicable.

Competing interests

The authors have no conflicts of interest to disclose.

Author contributions

Maíra Macedo Norões, Larissa Gonzaga Santos, Arthur da Silveira Prudente and Muryel de Carvalho Gonçalves performed the experimental procedures. Elaine Cristina Gavioli and Vanessa de Paula Soares Rachetti performed the literature searches and analyses. Janiana Raíza Jentsch Matias Oliveira wrote the first draft of the manuscript. Michel Fleith Otuki and Daniela de Almeida Cabrini contributed to writing, reviewing and editing. Delia Preti performed the synthesis of HC030031compound, Juliano Ferreira, Francesco De Logu, Romina Nassini and Eunice André defined the experimental design of the study.

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Figure Legends

Fig. 1 Ear oedema induced by volatile chemical irritants. Time-course of ear oedema induced by topical application xylene (100%/20 µl/ear) and toluene (100%/20 µl/ear) in mice. The effects of these chemical irritants were measured 30, 60, 90 and 120 min after the administration of chemical irritants and were expressed as the change in ear thickness. Each point represents the mean ± S.E.M. for 8 animals. . *P < 0.05 vs. vehicle (Student's t-test).

Fig. 2 Effect of TRPA1 receptor antagonist on the edematogenic responses induced by volatile chemical irritants. HC-030031 topical pretreatment was performed 15 min before the topical application of xylene (100%/20 µl/ear; A) or toluene (100%/20 µl/ear; B). Ear oedema was measured 30 min after administration of each chemical irritant and were expressed as the change in ear thickness. Each column represents the mean ± S.E.M. for 8 animals. *P < 0.05 vs. vehicle plus xylene or toluene (Veh_1) (one-way ANOVA followed by Bonferroni's test).

Fig. 3 Ear oedema induced by topical application of volatile chemical irritants in wild type (*Trpa1*$^{+/+}$) or *TRPA1*-deficient mice (*Trpa1*$^{-/-}$). Ear oedema was measured 30, 60, 90 and 120 min after the topical administration of xylene (100%/20 µl/ear; A) or toluene (100%/20 µl/ear; B) and are expressed as the change in ear thickness. Each point represents the mean ± S.E.M. for at least 5 animals. *P < 0.05 vs. *Trpa1*$^{+/+}$ (Student's t-test).

Fig. 4 Representative light microphotograph and graphic of the effects of TRPA1 antagonist on xylene and toluene-induced ear edema in mice. A thin section was obtained from ear tissue of mice pretreated with HC-030031 (300 nmol/20 µl/ear) or vehicle (acetone, 20 µl/ear) 30 min after the topical application of xylene (100%/20 µl/ear), toluene (100%/20 µl/ear) or vehicle (Veh, acetone, 20 µl/ear) (hematoxylin-eosin staining) x40. (A) Veh- Veh; (B) Veh-Xylene; (C) HC-030031-Xylene; (D) Veh-Toluene; (E) HC-030031-Toluene; (F) Representative graphic of light microphotograph of the effects of TRPA1 antagonist on xylene and toluene-induced ear edema in mice. Each column represents the mean ± S.E.M. for 8 animals. *P < 0.05 vs. xylene or toluene (one-way ANOVA followed by Bonferroni's test).

Fig. 5 Representative light microphotograph and graph indicating the effect of genetic deletion TRPA1 receptor on xylene-induced ear oedema in mice. Thin sections were obtained from the ear tissue of mice *Trpa1*$^{+/+}$ and *Trpa*$^{-/-}$30 min after the topical application of xylene or vehicle (Veh, 20 µl/ear) (haematoxylin-eosin staining ×40). *P <

0.05 vs. vehicle (veh), §P < 0.05 vs. xylene, (one-way ANOVA followed by Bonferroni's test).

Fig. 6 Representative light microphotograph and graph indicating the effect of genetic deletion TRPA1 receptor on toluene-induced ear oedema in mice. Thin sections were obtained from ear tissue of mice *Trpa1*$^{+/+}$ and *Trpa1*$^{-/-}$30 min after the topical application of toluene or vehicle (Veh, acetone, 20 µl/ear) (haematoxylin-eosin staining ×40). *P < 0.05 vs. vehicle (veh), §P < 0.05 vs. toluene, (one-way ANOVA followed by Bonferroni's test).

Fig. 7 Nociceptive responses induced by volatile chemical irritants. Nociceptive dose-response curves induced by topical application of xylene (30-100%/20 µl/paw; A) or toluene (30-100%/20 µl/paw; B) in the right hind paw of mice. Nociception time was assessed by recording the time spent evoking nociceptive responses during 5 min of observation immediately after exposure to chemical irritants. Each point represents the mean ± S.E.M. for 8 animals per group. *P< 0.05 compared to the xylene 30% or toluene 30% group (one-way ANOVA followed by Bonferroni's test).

Fig. 8 Effects of TRPA1 antagonist on the nociceptive responses produced by volatile chemical irritants. Effects of oral pretreatment with HC-030031 on the nociceptive responses induced by the topical administration of xylene (50%/20 µl/ear; A) or toluene (50%/20 µl/ear; B) in the hind paw of mice. Nociception time was assessed by recording the time spent evoking nociceptive responses during 5 min of observation immediately after exposure to chemical irritants. Each column represents the mean ± S.E.M. for at least 8 animals. *P < 0.05 vs. vehicle plus xylene or toluene (Veh_1) (Student's t-test).

Fig. 9 Nociceptive responses induced by the topical application of volatile chemical irritants in wild type (*Trpa1*$^{+/+}$) or *TRPA1*-deficient mice (*Trpa1*$^{-/-}$). Nociception time was measured during 5 min after the topical administration of xylene (50%/20 µl/paw; A) or toluene (50%/20 µl/paw; B) in the hind paw of mice. Each point represents the mean ± S.E.M. for 5 animals. *P < 0.05 vs. TRPA1 wild type mice (*Trpa1*$^{+/+}$) (Student's t-test).

Figure 1

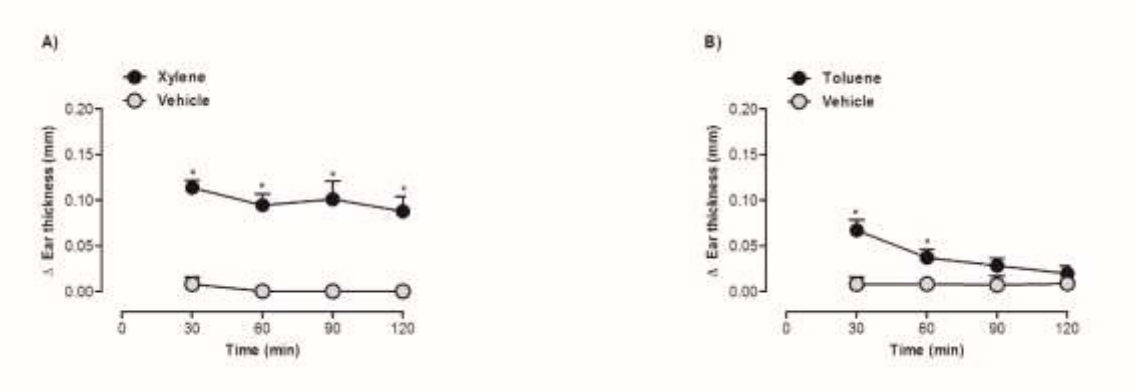

A)
Xylene
Vehicle
Δ Ear thickness (mm)
0.20
0.15
0.10
0.05
0.00
0
30
60
90
120
Time (min)
B)
Toluene
Vehicle
Δ Ear thickness (mm)
0.20
0.15
0.10
0.05
0.00
0
30
60
90
120
Time (min)

Figure 2

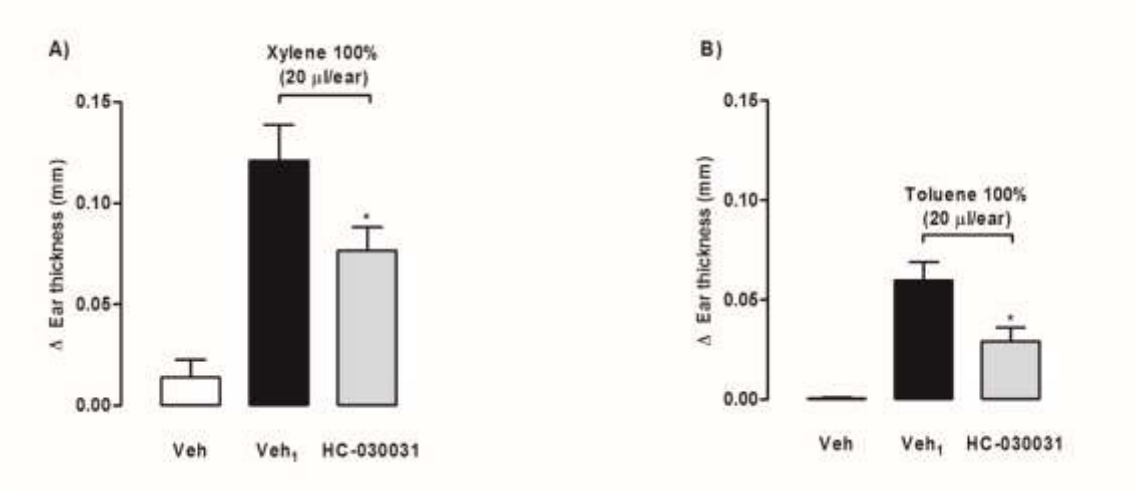

A)
Xylene 100%
(20 µl/ear)
0.15
0.10
0.05
0.00
Δ Ear thickness (mm)
*
Veh
Veh1
HC-030031
B)
0.15
0.10
0.05
0.00
Δ Ear thickness (mm)
Toluene 100%
(20 µl/ear)
*
Veh
Veh1
HC-030031

Figure 3

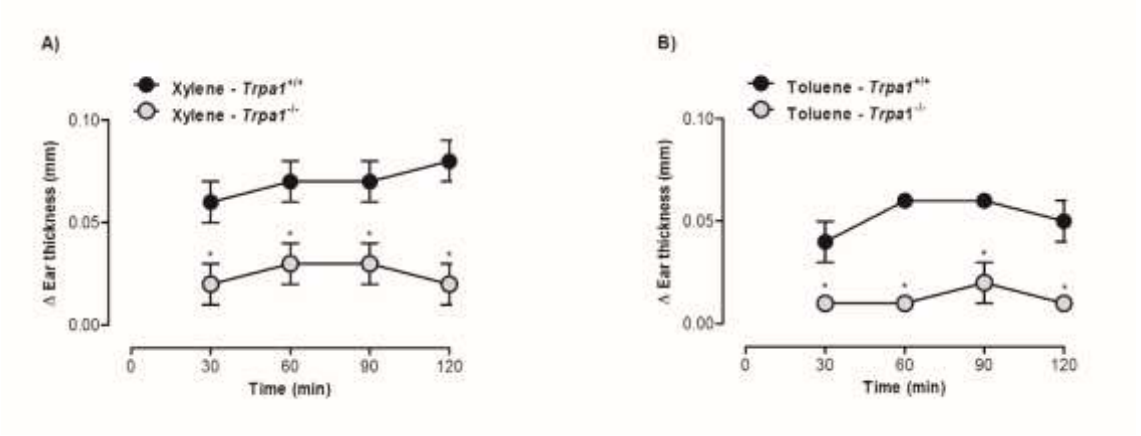

A)
Xylene - Trpa1+/+
Xylene - Trpa1-/-
Δ Ear thickness (mm)
0.10
0.05
0.00
0
30
60
90
120
Time (min)
B)
Toluene - Trpa1+/+
Toluene - Trpa1-/-
Δ Ear thickness (mm)
0.10
0.05
0.00
0
30
60
90
120
Time (min)

Figure 4

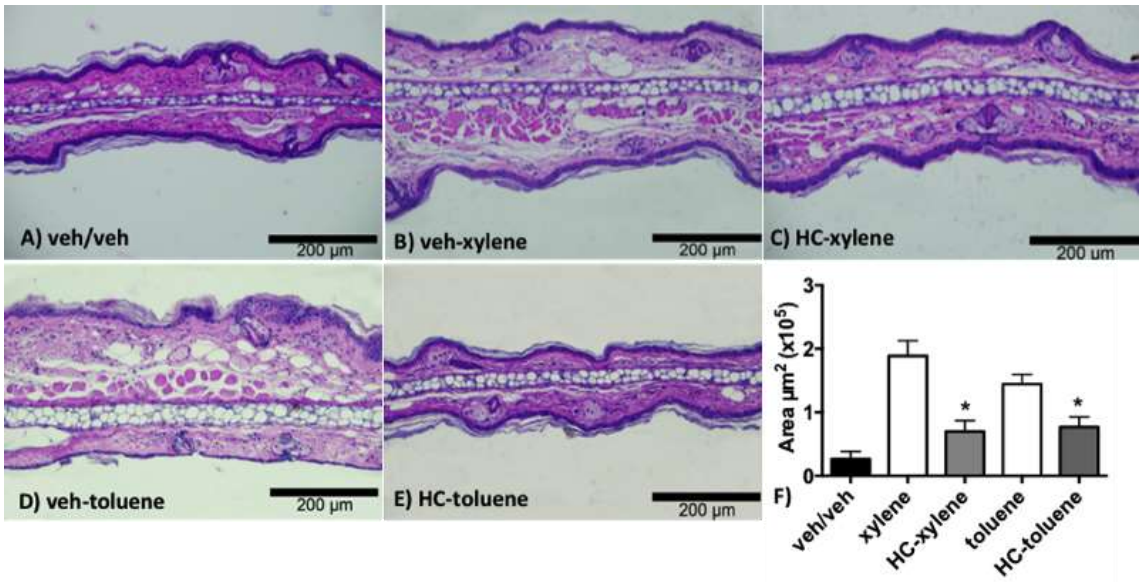

A) veh/veh
200 µm
B) veh-xylene
200 µm
C) HC-xylene
200 µm
D) veh-toluene
200 µm
E) HC-toluene
200 µm
F)
Area µm² (x10⁵)
3
2
1
0
*
*
veh/veh
xylene
HC-xylene
toluene
HC-toluene

Figure 5

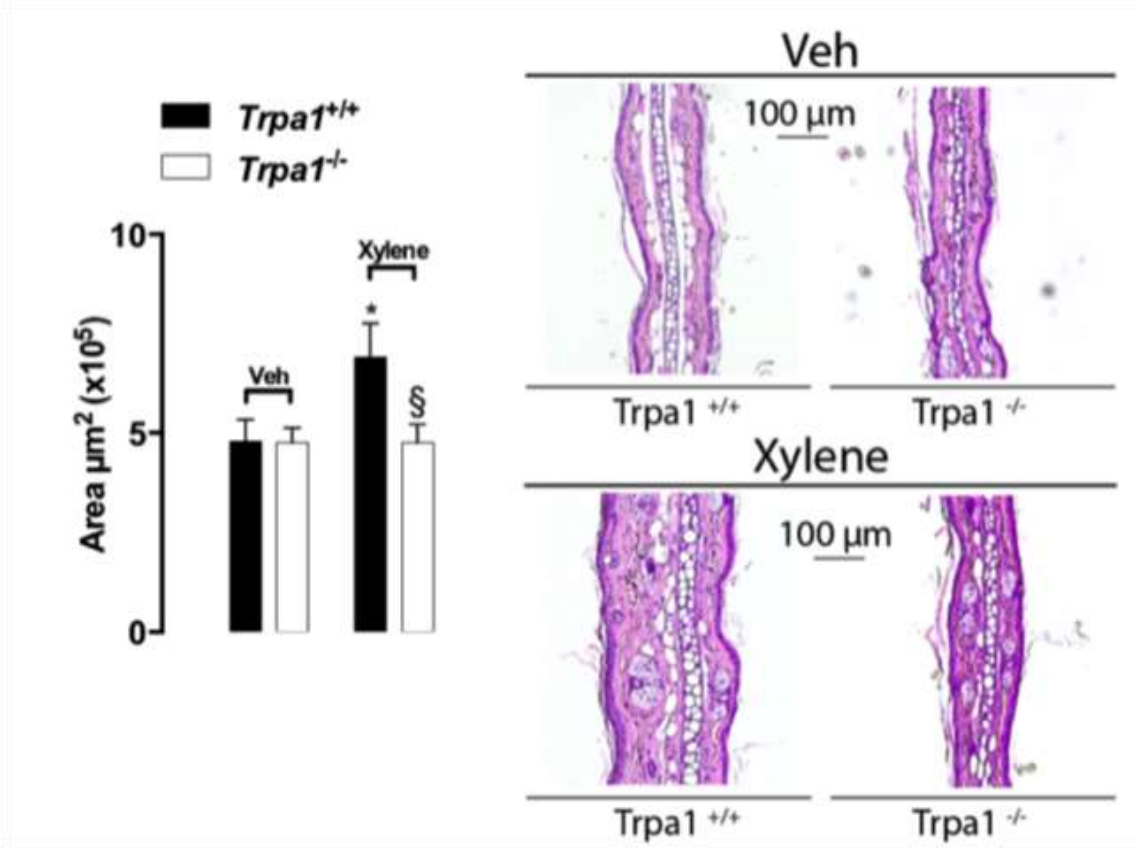

Trpa1+/+
Trpa1-/-
Area µm2 (x105)
10
5
0
Veh
Xylene
*
§
Veh
100 µm
Trpa1 +/+
Trpa1 -/-
Xylene
100 µm
Trpa1 +/+
Trpa1 -/-

Figure 6

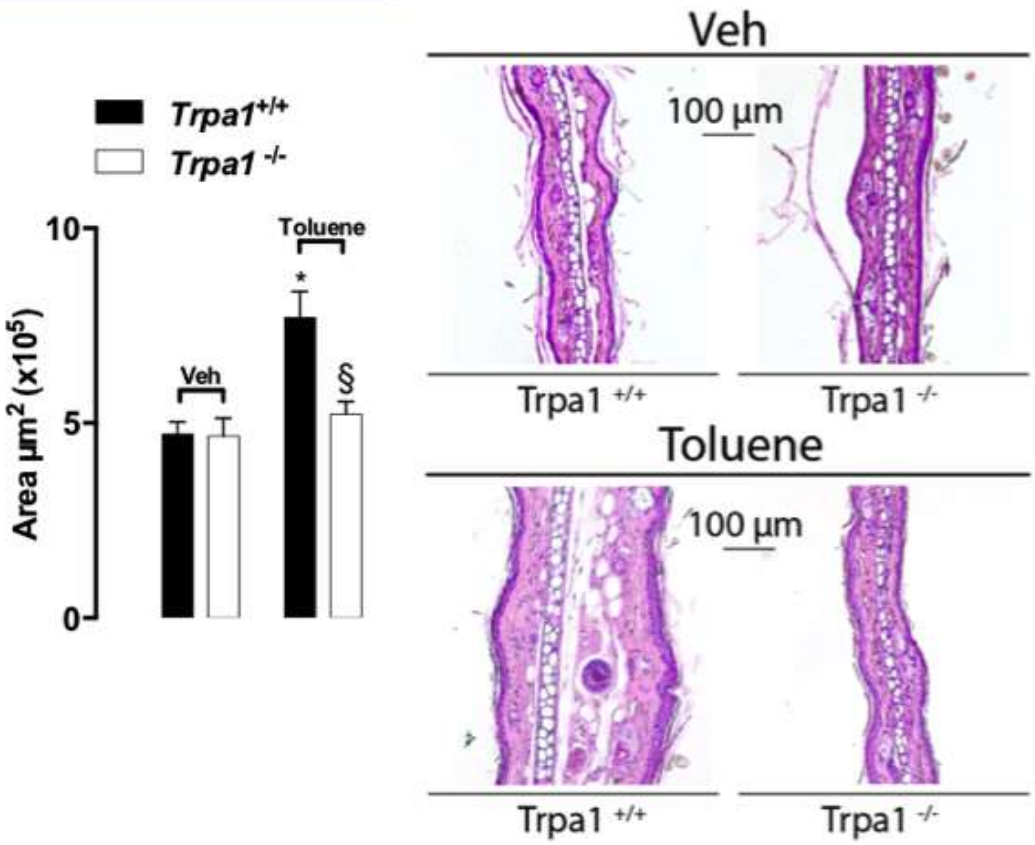

Trpa1+/+
Trpa1 -/-
Area µm2 (x10^5)
10
5
0
Veh
Toluene
*
§
Veh
100 µm
Trpa1 +/+
Trpa1 -/-
Toluene
100 µm
Trpa1 +/+
Trpa1 -/-

Figure 7

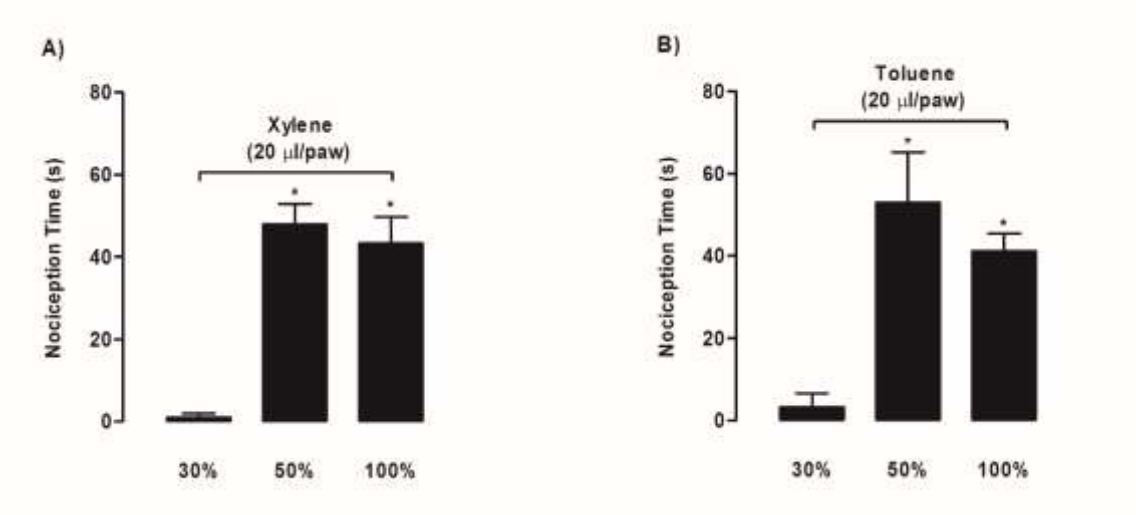

A)
Xylene
(20 µl/paw)
Nociception Time (s)
80
60
40
20
0
30%
50%
100%
B)
Toluene
(20 µl/paw)
Nociception Time (s)
80
60
40
20
0
30%
50%
100%

Figure 8

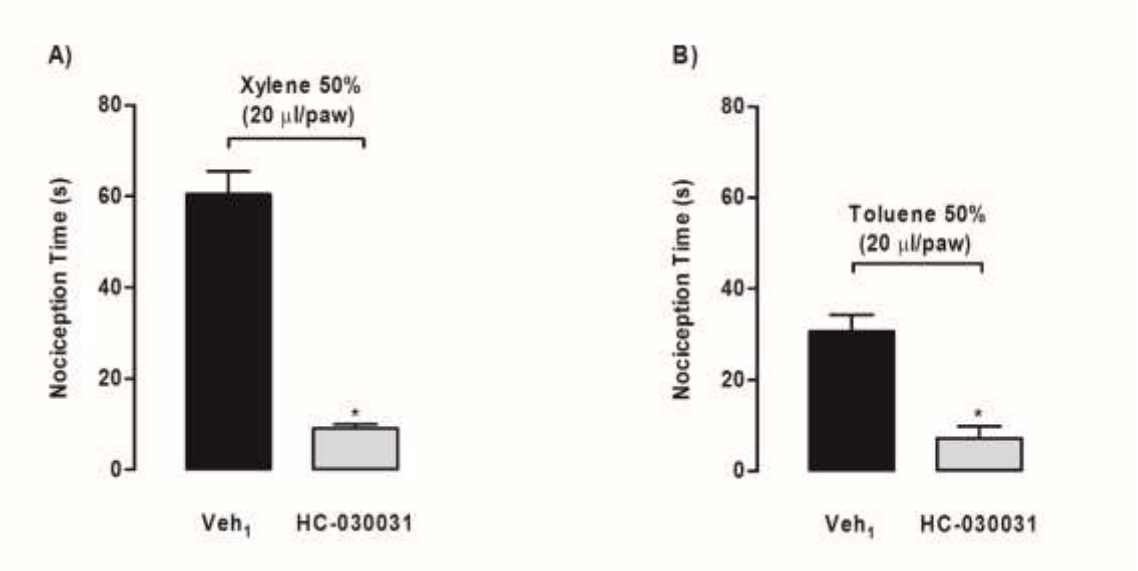
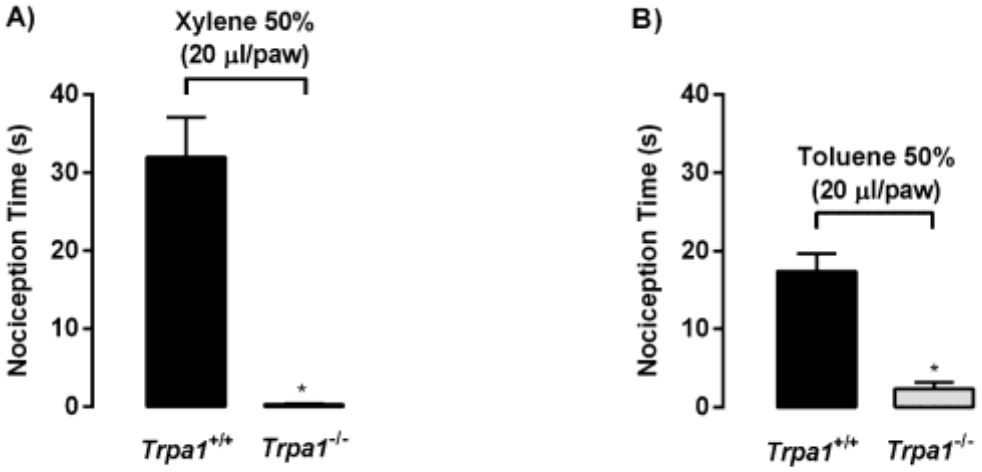

A)
Xylene 50%
(20 µl/paw)
Nociception Time (s)
80
60
40
20
0
*
Veh1
HC-030031
B)
Toluene 50%
(20 µl/paw)
Nociception Time (s)
80
60
40
20
0
*
Veh1
HC-030031

Figure 9


A)
Xylene 50%
(20 µl/paw)
Nociception Time (s)
40
30
20
10
0
*
Trpa1+/+
Trpa1-/-
B)
Toluene 50%
(20 µl/paw)
Nociception Time (s)
40
30
20
10
0
*
Trpa1+/+
Trpa1-/-