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RESEARCH PAPER

The blockade of transient receptor potential ankirin 1 (TRPA1) signalling mediates antidepressant- and anxiolytic-like actions in mice

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BACKGROUND AND PURPOSE

Transient receptor potential vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) are involved in many biological processes, including nociception and hyperalgesia. Whereas the involvement of TRPV1 in psychiatric disorders such as anxiety and depression has been reported, little is known regarding the role of TRPA1 in these conditions.

EXPERIMENTAL APPROACH

We investigated the role of TRPA1 in mice models of depression [forced swimming test (FST)] and anxiety [elevated plus maze (EPM) test].

KEY RESULTS

Administration of the TRPA1 antagonist (HC030031, 30 nmol in 2 μL, i.c.v.) reduced immobility time in the FST. Similar results were obtained after oral administration of HC030031 (30–300 mg·kg^{-1}). The reduction in immobility time in FST induced by HC030031 (100 mg·kg^{-1}) was completely prevented by pretreatment with TRPA1 agonist, cinnamaldehyde (50 mg·kg^{-1}, p.o.), which *per se* was inactive. In the EPM test, pretreatment with cinnamaldehyde (50 mg·kg^{-1}, p.o.), which *per se* did not affect behaviour response, prevented the anxiolytic-like effect (increased open arm exploration) evoked by TRPA1 blockade (HC030031, 100 mg·kg^{-1}, p.o.). Treatment with either cinnamaldehyde or HC030031 did not affect spontaneous ambulation. Furthermore, TRPA1-deficient mice showed anxiolytic- and antidepressant-like phenotypes in the FST and EPM test respectively.

CONCLUSION AND IMPLICATIONS

The present findings indicate that genetic deletion or pharmacological blockade of TRPA1 produces inhibitory activity in mouse models of anxiety and depression. These results imply that TRPA1 exerts tonic control, promoting anxiety and depression, and that TRPA1 antagonism has potential as an innovative strategy for the treatment of anxiety and mood disorders.

Abbreviations

ECC, European Communities Council; EPM, elevated plus maze; FST, forced swimming test; PAG, periaqueductal grey; TRPA1, transient receptor potential ankyrin 1; *Trpa1*$^{-/-}$, transient receptor potential ankyrin 1 deficient mice; *Trpa1*$^{+/+}$, transient receptor potential ankyrin 1 wild-type mice; TRPC6, transient receptor potential canonical 6; TRPV1, transient receptor potential vanilloid 1; TRPV3, transient receptor potential vanilloid 3; TRPV4, transient receptor potential vanilloid 4

Table of Links

TARGETS	LIGANDS
TRPA1	cinnamaldehyde
TRPC6	diazepam
TRPV1	nortriptyline
TRPV3	
TRPV4	
TRPM8	

This Table lists protein targets and ligands which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013).

Introduction

Transient receptor potential (TRP) ion channels, including TRPV1 (transient receptor potential vanilloid 1), TRPV3 (transient receptor potential vanilloid 3), TRPV4 (transient receptor potential vanilloid 4), TRP ankyrin 1 (TRPA1) and the TRP melastatin 8 (TRPM8), have attracted attention for their role in a variety of sensory processes. Additional localizations and functions have been proposed for TRP channels, and in particular, for those mainly expressed by nociceptors. As an example, the capsaicin 'receptor' TRPV1 (Caterina *et al.*, 1997; Caterina and Julius, 2001; Szallasi *et al.*, 2007) has been reported in the CNS, including the prefrontal cortex, hippocampus, amygdala, hypothalamus, periaqueductal grey (PAG), locus coeruleus and cerebellum (Mezey *et al.*, 2000; Roberts *et al.*, 2004; Toth *et al.*, 2005). This widespread presence in the CNS has led to the suggestion that TRPV1 is involved in the control of emotions, such as anxiety and mood (Aguiar *et al.*, 2009; Campos and Guimaraes, 2009). In rats, pharmacological TRPV1 blockade produces anxiolytic-like effects in the elevated plus maze (EPM) and Vogel tests (Santos *et al.*, 2008; Terzian *et al.*, 2009). Other studies demonstrated that TRPV1 activation facilitates long-term potentiation, suppresses long-term depression and inhibits excitatory synapses in hippocampal slices of rats, thus suggesting a role in epileptogenesis (Li *et al.*, 2008; Fu *et al.*, 2009). Antidepressant-like behavioural effects were also observed in TRPV1-deleted mice in the novelty-suppressed feeding test and in the forced swimming test (FST) (You *et al.*, 2012). TRPV1-deleted mice also exhibited increased aggressiveness and reduced social interactions in social dominance and social interaction tests respectively (You *et al.*, 2012). Additional TRPs, such as TRPC6 (transient receptor potential canonical 6) (Leuner *et al.*, 2007; Morelli *et al.*, 2013), have been proposed to contribute to CNS functions.

The TRPA1 channel, which has also been shown to contribute to nociception and hyperalgesia (Baraldi *et al.*, 2010; Kaneko and Szallasi, 2013), is activated by noxious cold temperatures and by a variety of reactive molecules of alimentary origin, including allyl isothiocyanate from mustard oil, cinnamaldehyde from cinnamon, allicin from garlic (Bandell *et al.*, 2004; Macpherson *et al.*, 2005), produced by environmental processes as acrolein (Bautista *et al.*, 2006) or in tobacco smoke as crotonaldehyde (Andre *et al.*, 2008), and more importantly, by an unprecedented series of endogenously produced reactive oxygen and nitrogen species and their by-products generated by peroxidation and nitrosylation of plasma membrane phospholipids (Bautista *et al.*, 2006; Trevisani *et al.*, 2007; Andersson *et al.*, 2008; Materazzi *et al.*, 2008; Taylor-Clark *et al.*, 2008; Baraldi *et al.*, 2010).

Although the classical localization of TRPA1 is represented by nociceptive neurons (Story *et al.*, 2003), functional TRPA1 has been reported in a large variety of cells, including skin keratinocytes (Atoyan *et al.*, 2009; Biro and Kovacs,

2009), hair, urinary bladder (Nagata *et al.*, 2005) and airways/lung epithelial cells (Streng *et al.*, 2008; Nassini *et al.*, 2012), and other cells (Szallasi, 2013). In the rat CNS, localization of TRPA1 was only investigated in trigeminal sensory nuclei, the superficial laminae of the trigeminal caudal nucleus, spinal dorsal horn, and nucleus tractus solitarii neurons where a few axons and terminals have been demonstrated immunopositive for TRPA1 (Sun *et al.*, 2009; Kim *et al.*, 2010). Little information exists on the expression of TRPA1 receptors in the brain. However, in the dog brain, cerebellum TRPA1 mRNA has been found abundantly (Doihara *et al.*, 2009).

Considering the role proposed for TRPV1 receptors in anxiety- and mood-related behaviours, and the possible localization of TRPA1 in the CNS, we investigated whether TRPA1 receptor modulates depression and anxiety. To achieve this goal, the behavioural tests widely used for investigating the effects of antidepressant and anxiolytic drugs were applied to mice treated with TRPA1 receptor ligands and in TRPA1-deleted mice.

Methods

Animals

Male Swiss mice 60 days old (weighing between 30 and 35 g) obtained from the Federal University of Rio Grande do Norte, Brazil, breeding colony were used in the pharmacological studies. Male wild-type (*Trpa1*$^{+/+}$) or TRPA1-deficient (*Trpa1*$^{-/-}$B6;129P-Trpa1tm1Kykw/J) mice generated by heterozygous animals on a C57BL/6 background (Jackson Laboratories, Bar Harbor, ME, USA) were used in the phenotypic characterization studies. We have compared the behaviour of these animals (Swiss vs. C57BL/6) and we found that these responses were essentially the same in the different strains of mice.

Mice were kept under standard animal housing conditions (12 h light/dark cycle, lights on at 06:00 h, with food and water *ad libitum* (maximum of 20 mice group-housed). All pharmacological experiments were conducted in accordance with the standards set in the directives for animal care of Brazilian law No. 11.794/2008, with the approval of the local Ethics Committee (Protocol No. 013/2010), while experiments with *Trpa1*$^{+/+}$ or *Trpa1*$^{-/-}$ mice were carried out in accordance with the European Communities Council (ECC) guidelines for animal care procedures and Italian legislation (DL 116/92) application of the ECC directive 86/609/EEC. Studies were conducted under the University of Florence research permit No. 143/2008-B and No. 204/2012-B. The minimum number of animals required to obtain consistent data were employed. The animals employed in this study were used only once. Mice were randomly assigned before treatment or behavioural phenotype evaluation. This study strictly followed the ARRIVE guidelines as previously reported by Kilkenny *et al.* (2010).

Procedure for i.c.v. administration

An 8 mm guide cannula (25 × 0.7 mm) had to be stereotaxically implanted for i.c.v. injections; it was placed in the left lateral ventricle, according to the following coordinates from bregma: AP: –0.6 mm, ML: +1.1 mm, DV: –1.0 mm (Paxinos and Franklin, 2008). Surgery occurred under ketamine-xylazine anaesthesia (100 and 10 mg·kg^{-1}, i.p. respectively). The cannula was secured to the skull by acrylic dental cement. Immediately after cannula implantation, animals were treated with diclofenac sodium (10 mg·kg^{-1}, s.c.) and oxytetracycline (20 mg·kg^{-1}, i.m.). Four to five days after surgery, HC030031 (30 nmol) or grape oil 100% were administered i.c.v. in a volume of 2 µL. The injection was made at the rate of 2 µL·min^{-1} controlled by an automatic micropump infuser (Insight Equipamentos, Ribeirão Preto, Brazil) through a needle (27 G) protruding 1 mm from the cannula tip, which was connected by a polyethylene tube (PE20) to a Hamilton microsyringe. Five minutes after the i.c.v. administration, animals were subjected to the FST. After the behavioural test, animals were killed with thiopental (100 mg·kg^{-1}, i.p.), and methylene blue (2µL) was injected through the cannula. Animals without dye in the lateral ventricle were discarded from the statistical analysis (approximately 5% of animals).

Behavioural tests

FST. The FST was performed as previously described (Porsolt *et al.*, 1977). Briefly, mice were dropped individually into a glass cylinder (height: 25 cm; diameter: 15 cm) containing 18 cm of water, maintained at 23–25°C, and left there for 6 min. A mouse was judged to be immobile when it floated in an upright position, and made only small movements to keep its head above water. To validate our experimental conditions, the effect of nortriptyline (at distinct doses), i.p. injected 60 min before the test, was evaluated. The duration of immobility was recorded during the last 4 min of the 6 min testing time period. Experiments were performed in the light cycle between 13:00 and 17:00 h.

EPM test. The EPM test consisted of two open (30 × 5 cm) and two wall-enclosed arms (30 × 5 × 15 cm) connected by a central platform (5 × 5 cm) as previously described (Lister 1987). The apparatus was elevated 40 cm above the floor. The floor and the walls of the enclosed arms of the maze were constructed of brown wood. Each mouse was placed on the central platform, facing a closed arm and observed for a 5 min time period. The frequency of entry into either open or enclosed arms, as well as the time spent in each arm type, was recorded (in seconds). An entry was scored as such only when the animal placed all four limbs into any given arm. Drugs with anxiolytic-like activity usually increase the time spent in and/or frequency of entries into open arms, whereas the reverse holds true for anxiogenic-like drugs. In this study, the effect of 1 mg·kg^{-1} (i.p.) diazepam, 15 min before the behavioural test, was used for validating our experimental conditions. Furthermore, the number of entries into closed arms was used as an index of general activity. The EPM apparatus was placed in a small closed room lit by a red light, with an intensity of 100 lux in the open arms. After each mouse, the apparatus was cleaned with 5% ethanol. EPM test was recorded in the light cycle between 13:00 and 17:00 h.

Open field test. The open field consisted of a square arena (60 × 60cm), made of white wood with a plastic black floor surrounded by 60 cm high walls. A video camera was positioned above the apparatus and was connected to a computer.

Experiments were recorded and automatically analysed using the video tracking AnyMaze Software (Stoelting Co, Wood Dale, IL, USA). These experiments were performed in a calm and red illuminated room. The animals were gently placed on the center of the arena and they were allowed to explore the apparatus individually during a 30 min time period. The total distance travelled (m) by each animal was averaged into 5 min time bins. After the behavioural evaluation of each mouse, the arena was cleaned with 5% ethanol solution. Locomotor activity was recorded in the light cycle between 13:00 and 17:00 h.

Experimental design

Effects of the oral, i.c.v. administration of TRPA1 ligands and of genetic deletion of TRPA1 receptor on the behaviour of mice in the FST. In order to evaluate the contribution of TRPA1 receptors to mouse behaviour, the selective TRPA1 antagonist HC030031 (McNamara *et al.*, 2007) was injected i.c.v. (30 nmol in 2 μL, 5 min i.c.v. min before the behavioural test; da Costa *et al.*, 2010) or administered p.o. at doses of 30, 100 and 300 mg·kg^{-1}, by employing a gavage needle, in a volume of 10 mL·kg^{-1}, 90 (p.o.) min before the behavioural test. Similarly, the TRPA1 agonist, cinnamaldehyde (50 mg·kg^{-1}, p.o.), was also administered 90 min before the behavioural test. In addition, mice were pretreated with cinnamaldehyde (50 mg·kg^{-1}, p.o.) 15 min before the administration of HC030031 (100 mg·kg^{-1}, p.o.), and the animal's behaviour was assessed 90 min after the last injection. The treatment with a classic antidepressant, nortriptyline (30 mg kg^{-1}, i.p.), was made 60 min before starting behavioural evaluations, and the doses used were based on those used in a previous study (Reny-Palasse *et al.*, 1989). Control groups were treated with the same vehicle used to dissolve the drugs. All experimental procedures, including control groups, followed the same schedule described for the treated groups. In another set of experiments, *Trpa1*$^{+/+}$ or *Trpa1*$^{-/-}$ mice were subjected to the FST. Behavioural observations were made by an experienced observer who was blind with respect to the treatment conditions. All experimental procedures, including vehicle treatment, followed the same schedule described for the treated groups.

Effects of oral administration of TRPA1 ligands and of genetic deletion of TRPA1 receptor on the behaviour of mice in the EPM test. In order to evaluate the effect of the administration of agonist or antagonist of TRPA1 receptors on mouse behaviour, HC030031 (100 mg·kg^{-1}, p.o.) or cinnamaldehyde (50 mg·kg^{-1}, p.o.) was administered, and the behaviour of animals in the EPM test was assessed 90 min after drug injection. In addition, mice were pretreated with cinnamaldehyde (50 mg·kg^{-1}, p.o.) 15 min before the administration of HC030031 (100 mg·kg^{-1}, p.o.), and the behaviour of animals was assessed 90 min after the last administration. In another set of experiments, *Trpa1*$^{+/+}$ or *Trpa1*$^{-/-}$ mice were subjected to the EPM test. Control groups were treated with the same vehicle employed to dissolve the drugs. All experimental procedures, including vehicle treatment, followed the same schedule described for treated groups. Behavioural observations were made by an experienced observer who was blind with respect to the treatment conditions.

Effects of oral administration of TRPA1 ligands and of genetic deletion of TRPA1 receptor on the behaviour of mice in the open field test. To evaluate the effect of TRPA1 receptor ligands on spontaneous locomotor activity, the animals were subjected to the open field test. Either HC030031 (100 mg·kg^{-1}, p.o.) or cinnamaldehyde (30 and 50 mg·kg^{-1}, p.o.) was administered 90 min before the behavioural test and the behaviour of the animals was assessed in the open field test. Vehicle groups were treated with the same vehicle employed to dissolve the drugs.

In another set of experiments, *Trpa1*$^{+/+}$ or *Trpa1*$^{-/-}$ mice were subjected to the open field test.

Statistical analysis

Results were analysed by INSTAT (version 3.06; GraphPad Software Inc., San Diego, CA, USA) and Statistica (version 5.1; StatSoft Inc., Tulsa, OK, USA). Pharmacological data are presented as mean ± SEM, and each value reflects the mean of 8–10 animals per group for the FST and EPM test, and 6–8 animals per group for the open field test. In the behavioural assessment of TRPA1-genetically modified mice in the EPM, FST and open field tests, at least 5 animals per group were employed. Data sets were initially checked for normality and homogeneity of variance before use of parametric statistical tests. Differences among experimental groups were determined by one-way ANOVA or two-way ANOVA followed by Student–Newman–Keuls *post hoc* test. For evaluating the effects of diazepam, HC030031 or cinnamaldehyde in the EPM test and HC030031 i.c.v. administered in the FST, differences among groups were analysed by Student's *t*-test. Differences were considered significant when P was less than 0.05.

Drugs

The TRPA1 antagonist (HC030031) was synthesized and purified as previously described (Andre *et al.*, 2008), whereas the TRPA1 agonist (cinnamaldehyde), nortriptyline, a standard antidepressant drug, and diazepam, a standard anxiolytic drug, were purchased from Sigma (St. Louis, MO, USA). HC030031 was suspended in 0.5% methylcellulose (for p.o.administration) or grape oil 100% (for i.c.v. administration) obtained from Sigma. Cinnamaldehyde was dissolved in saline (NaCl 0.9%) containing 3% of Tween 80 plus 10% DMSO. Nortriptyline was dissolved in saline, whereas diazepam was dissolved in saline and 0.5% Tween 80. All drugs were dissolved just before use. The range of doses used for cinnamaldehyde and HC030031 was chosen based upon previously published studies (Eid *et al.*, 2008; Masamoto *et al.*, 2009).

Results

Effect of treatment with TRPA1 ligands and genetic deletion of TRPA1 receptor on the mouse FST

Firstly, to validate the FST under our experimental conditions, mice were i.p. treated with nortriptyline (at distinct doses), a classical antidepressant, and then the effects on the FST were assessed. As expected, nortriptyline dose-dependently reduced the duration of immobility when compared with the control group [Figure 1A; $F_{(4,43)}$ = 16.56, P =

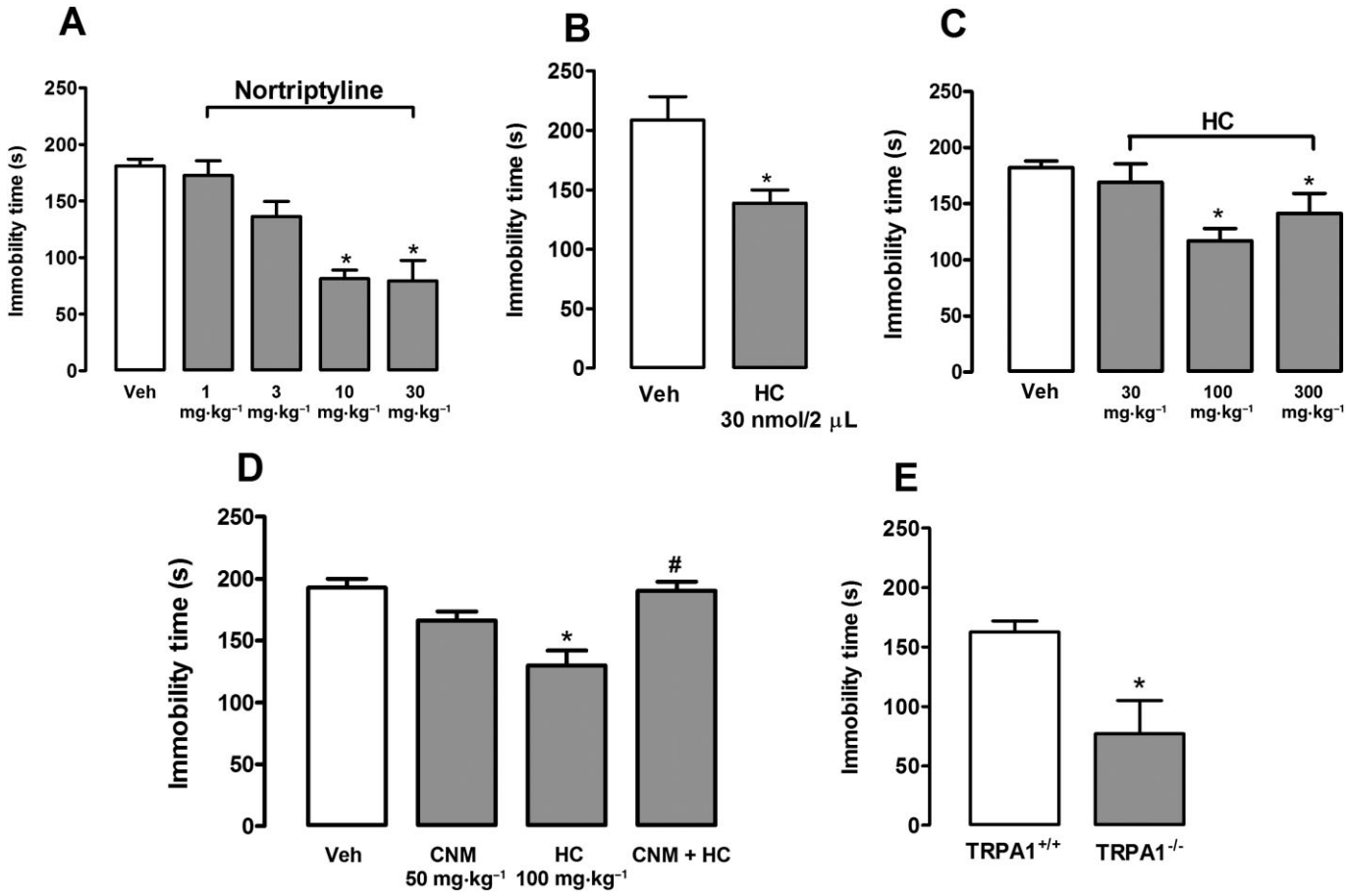

Figure 1

Effects of nortriptyline (3–30 mg·kg^{-1}, i.p.) (A), TRPA1 antagonist HC030031 (30 nmol in 2 µL, i.c.v.) (B), HC030031 (30, 100 and 300 mg·kg^{-1}, p.o.) (C), pretreatment with cinnamaldehyde (50 mg·kg^{-1}, p.o.) on reduction of immobility time induced by TRPA1 antagonist (HC030031, 100 mg·kg^{-1}, p.o.) (D) and genetic deletion of TRPA1 (E) on the immobility time in the FST in mice. Each column is presented as mean ± SEM of at least 7 animals per group or 5 animals per group with genetic deletion of TRPA1.*P < 0.05 versus vehicle values and #P < 0.05 versus HC030031 values (one- or two-way ANOVA followed by Student–Newman–Keuls test or Student's t-test).

0.0001]. Next, to test our initial hypothesis, the effect of the TRPA1 antagonist, HC030031, in the CNS was evaluated in the FST. We observed that the central injection of HC030031 (30 nmol/2 µL, i.c.v.) significantly reduced immobility time in mice (Figure 1B; t_8 = 2.4; P = 0.038). To support our observation, we also tested the oral administration of HC030031 at different doses (30, 100 and 300 mg·kg^{-1}, p.o.). We observed that the lower dose administered did not alter the duration of immobility time in the FST when compared with the control group. However, at higher doses (100 and 300 mg·kg^{-1}, p.o.) HC030031 significantly reduced immobility time in mice [Figure 1C; $F_{(3,49)}$ = 7.44, P = 0.0001].

Two-way ANOVA revealed a significant interaction between the treatment with HC030031 and cinnamaldehyde in the mouse immobility time [Figure 1D; $F_{(1,40)}$ = 20.20; P = 0.00006]. *Post hoc* analysis indicated that the oral administration of HC030031 (100 mg·kg^{-1}, p.o.), but not cinnamaldehyde 50 mg·kg^{-1} (Figure 1D; P > 0.05), reduced immobility time compared with vehicle-treated mice (Figure 1D; P < 0.05). Additionally, the reduction of immobility time induced by the treatment with HC030031 (100 mg·kg^{-1}, p.o.) was completely prevented by pretreatment with cinnamaldehyde (50 mg·kg^{-1}, p.o.) (Figure 1D).

In another experimental setting, it was observed that animals with genetic deletion of TRPA1 receptor displayed a significant reduction of immobility time compared with *Trpa1*$^{+/+}$ mice in the FST (Figure 1E; t_8 = 2.90; P = 0.02).

Effect of treatment with TRPA1 ligands and genetic deletion of TRPA1 receptor on the mouse EPM test

To better define our current experimental conditions in the EPM test, we first used the standard anxiolytic drug, diazepam (1 mg·kg^{-1}). Administration of diazepam increased the time spent in the open arms (Figure 2A; t_8 = 7.3, P = 0.0001), without affecting the frequency of entries into closed arms of the EPM test (Figure 2B; P > 0.05). Mice treated with HC030031 (100 mg·kg^{-1}, p.o.), but not cinnamaldehyde (50 mg·kg^{-1}, p.o.), displayed a significant increase of time spent in the open arms compared with control mice

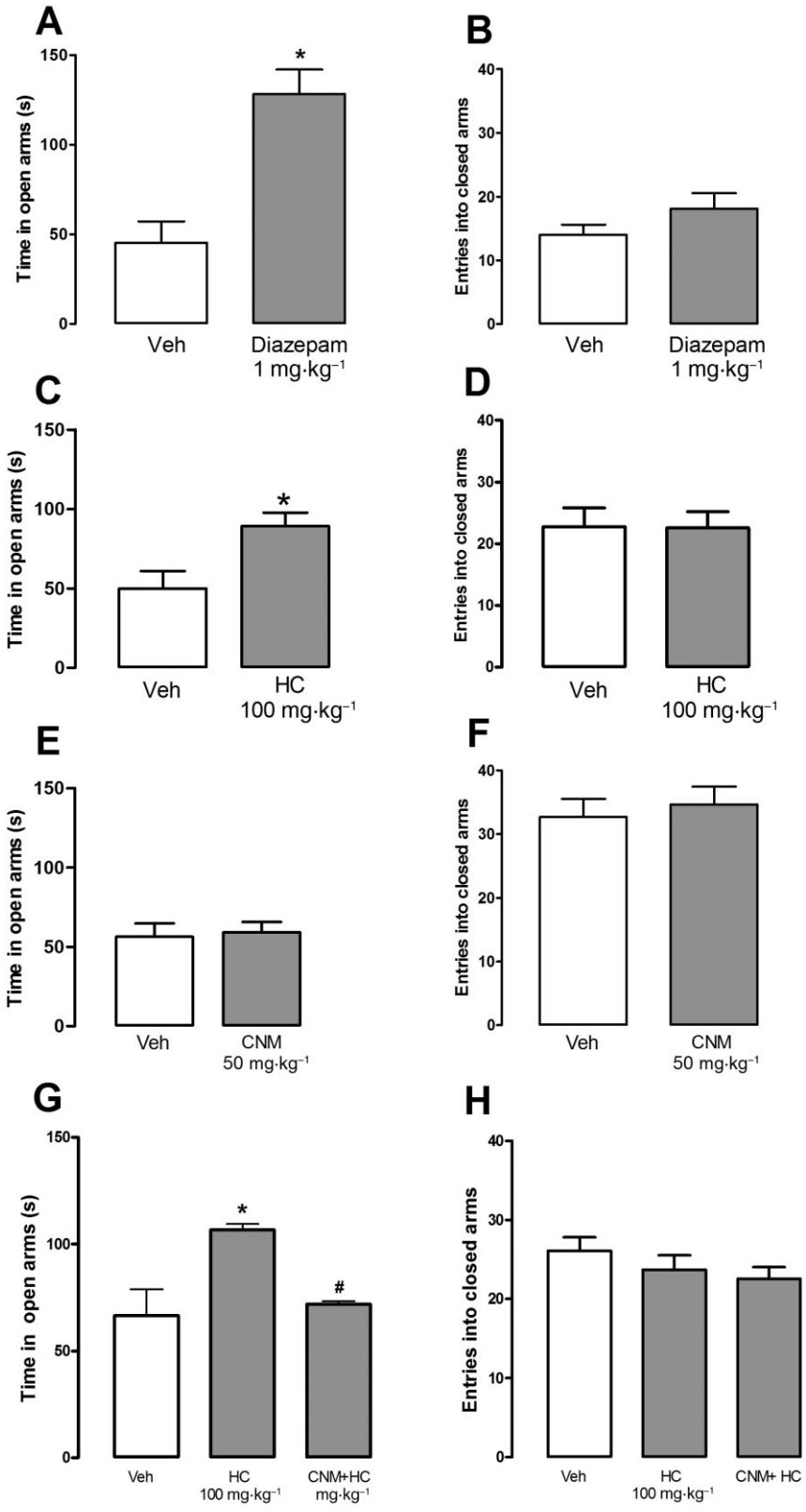

Figure 2

Effects of the standard anxiolytic diazepam (1 mg·kg^{-1}, i.p.) (A), HC030031 (100 ·mg kg^{-1}, p.o.) (C), cinnamaldehyde (50 mg·kg^{-1}, p.o.) (E), cinnamaldehyde (50 mg·kg^{-1}, p.o.) on mice treated with HC030031 (100 mg·kg^{-1}, p.o.) (G) on the time spent in open arms and on the frequency of entries into closed arms (B, D, F and H, respectively) in the EPM test in mice. Each column is presented as mean ± SEM of at least 9 animals per group.*$P < 0.05$ versus vehicle values (Student's *t*-test or one-way ANOVA followed by Bonferroni's test) and #$P < 0.05$ versus HC030031 values.

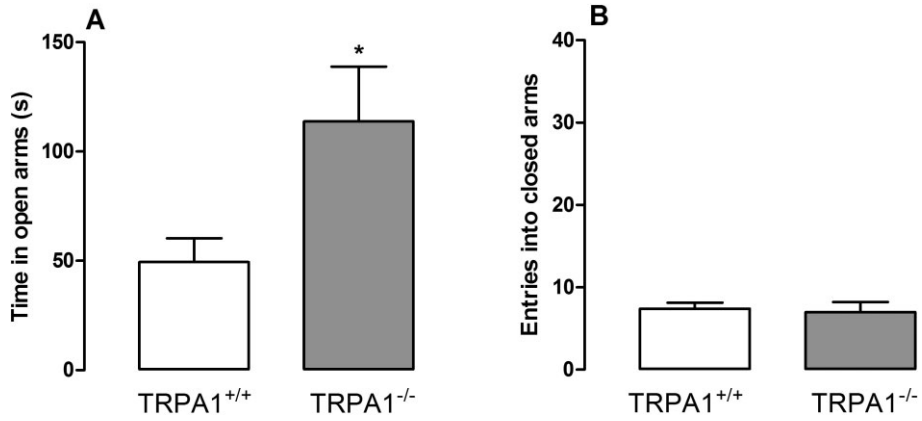

Figure 3

Behavioural phenotype of wild-type and knockout mice for the TRPA1 receptor in the EPM test. The behavioural parameters evaluated were the time spent in open arms (A), and the frequency of entries into closed arms (B) of the apparatus *$P < 0.05$ versus wild-type group (Student's *t*-test). Each column is presented as mean ±SEM of at least 5 animals per group.

(Figure 2C; $t_{14} = 2.83$, $P = 0.0135$; Figure 2E; $t_{13} = 0.28$, $P > 0.05$). Similar to diazepam, neither HC030031 nor cinnamaldehyde affected the frequency of entries into closed arms of the apparatus (Figure 2D; $t_{14} = 0.10$, $P > 0.05$; Figure 2F; $t_{13} = 0.68$, $P > 0.05$). Regarding the co-administration of HC030031 and cinnamaldehyde, the increased exploration to open spaces observed after HC030031 administration in the EPM test were completely prevented by cinnamaldehyde pretreatment (50 mg·kg^{-1}, p.o.) [Figure 2G; $F_{(2,25)} = 101.7$, $P < 0.001$] without affecting the frequency of entries into closed arms [Figure 2H; $F_{(2,25)} = 0.82$, $P > 0.05$]. In addition, we observed that *Trpa1*$^{-/-}$ displayed a significant increase in time spent in the open arms compared with *Trpa1*$^{+/+}$ mice in the EPM test (Figure 3A; $t_8 = 2,37$; $P < 0.05$) without affecting the frequency of entries into closed arms (Figure 3B; $t_8 = 0.49$; $P > 0.05$).

Effect of treatment with TRPA1 ligands and genetic deletion of TRPA1 receptor on mouse locomotor activity

We also evaluated the effects of treatment with HC030031 (100 mg·kg^{-1}, p.o.) and cinnamaldehyde (30 and 50 mg·kg^{-1}, p.o.) in the spontaneous locomotor activity during the first 5 min of observation (Figure 4A,C) and during 30 min of observation (Figure 4B,D) of mice in the open field test. As observed in our experiments, the administration of HC030031 and cinnamaldehyde did not significantly affect the general locomotor activity of mice ($P > 0.05$ for both treatments). Additionally, no significant differences in spontaneous ambulation were observed between *Trpa1*$^{-/-}$ compared to *Trpa1*$^{+/+}$ mice in the open field test (Figure 4E,F; $P > 0.05$).

Discussion

Results obtained in the current study indicate that the TRPA1 channel contributes to the modulation of depression- and anxiety-related behaviours in mice. This conclusion derives from the observation that pharmacological blockade of TRPA1 produced a pattern of effects similar to that obtained with the classical antidepressant and anxiolytic drugs nortriptyline and diazepam respectively. In particular, our findings show that the administration of the TRPA1 selective antagonist, HC030031, reduced immobility time in the mouse FST and increased the exploration of open arms in the EPM test. Results also demonstrated that effects evoked by TRPA1 antagonism were prevented by previous administration of the TRPA1 agonist, cinnamaldehyde. In addition, our findings show that neither TRPA1 blockade nor TRPA1 activation was able to affect spontaneous mice ambulation in the open field test. The absence of sedative/stimulant effects of either antagonism and agonism of the TRPA1 channel further supports the hypothesis that TRPA1 targeting results in a genuine influence on behavioural functions. In addition, Eid *et al.* (2008) demonstrated that HC030031 (100 mg·kg^{-1}) failed to have any effect during locomotor testing (rotarod), suggesting that HC030031, in this dose, does not affect the motor coordination of animals. Taken together, these results suggest the hypothesis that endogenous agonists acting on TRPA1 receptors exert a tonic control on behavioural responses favouring anxiety and depression in mice.

The absence of any effect by exogenous administered cinnamaldehyde represents an apparent contradictory finding. One possible explanation is that the TRPA1 channel tonically, and probably maximally, activated by putative endogenous agonist(s) cannot be further stimulated by the exogenous addition of cinnamaldehyde. In contrast, the effect of TRPA1 antagonism produced by HC-030031 can be overcome by administration of cinnamaldehyde, which re-activates channel function. The unique mode of activation of TRPA1 by a series of endogenously produced reactive molecules (Bautista *et al.*, 2006; Trevisani *et al.*, 2007) via a covalent, but reversible, reaction (Michael addition) (Macpherson *et al.*, 2005; Hinman *et al.*, 2006), could support this hypothesis. However, as there is no clear evidence for the endogenous modulation of TRPA1, the absence of any effect by cinnamaldehyde could be associated with the dose of cinna-

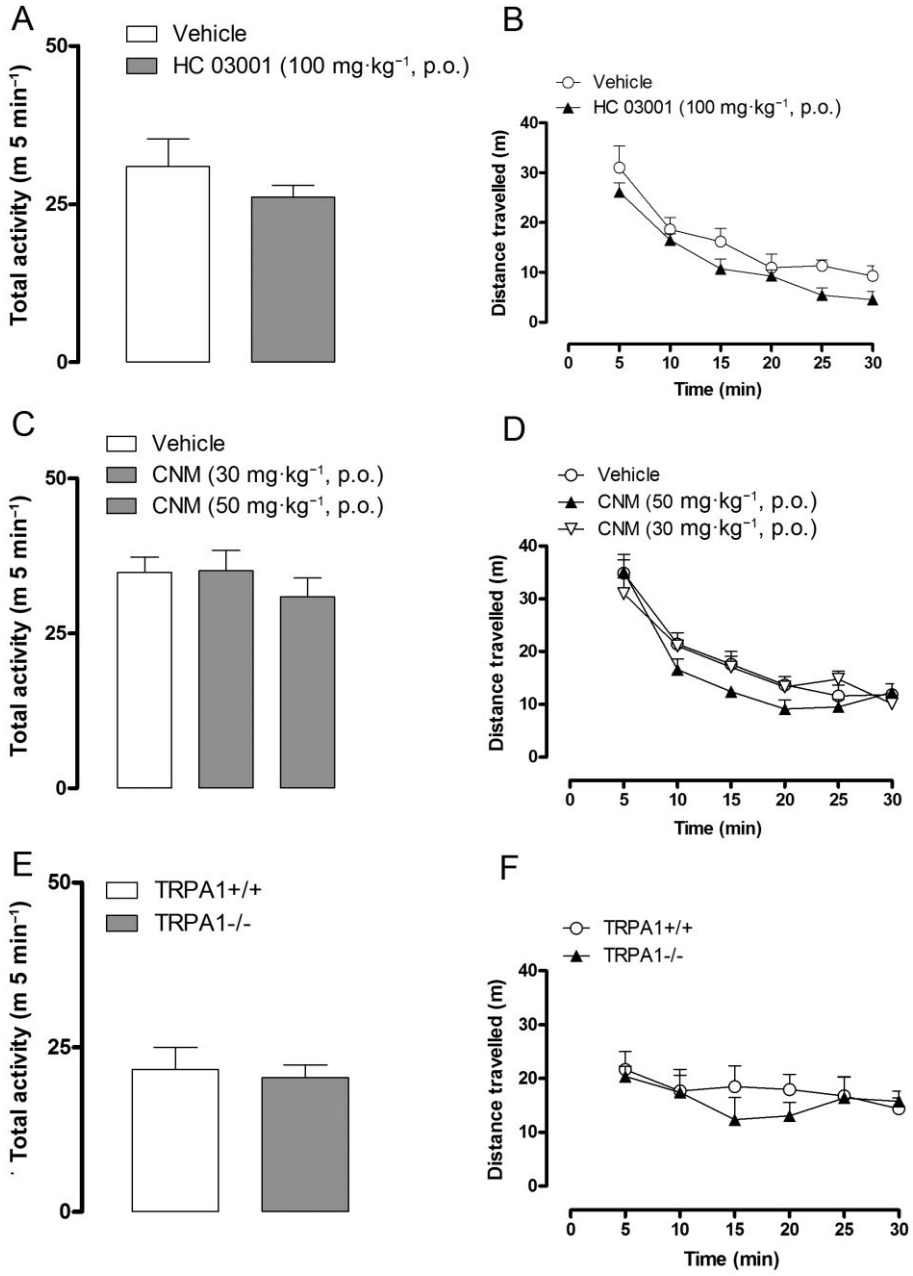

Figure 4

Effects of the treatment with HC030031 (100 mg·kg^{-1}, p.o.), cinnamaldehyde (30 and 50 mg·kg^{-1}, p.o.) and genetic deletion of TRPA1 receptor on the ambulation of mice in the open field test, shown as an average distance travelled in 5 min observation time (A, C, E). The locomotor activity during 30 min of observation in the open field is presented in panels B, D and F. Each column or point represents the mean of 5–8 animals per group and the vertical lines indicate the SEM.

maldehyde used in this study. We could not increase the dose of cinnamaldehyde because of their toxic effects (Masamoto *et al.*, 2009). Thus, this fact could illustrate the lack of effect of cinnamaldehyde *per se* in our study. Importantly, mice with genetic deletion of the TRPA1 receptor displayed an antidepressant and anxiolytic-like phenotype in the FST and EPM test, respectively, compared with wild-type mice. These findings markedly strengthen the hypothesis that ablation of TRPA1 signalling results in antidepressant- and anxiolytic-like behaviour in mice. However, we cannot rule out that phenotypic behaviour of TRPA1 knockout mice in the FST and EPM tests can be influenced by compensatory changes during development, and then, it may not reflect exclusively the absence of TRPA1 receptors.

Results from the literature have shown the expression of TRPV1 in different brain areas, such as prefrontal cortex, hippocampus, amygdala, hypothalamus, PAG, locus coeruleus and cerebellum, and some evidence implies a role for

TRPV1 in brain-related disorders (Li *et al.*, 2008; Aguiar *et al.*, 2009; Campos and Guimaraes, 2009; Fu *et al.*, 2009). TRPV1 blockade reduces the frequency of spontaneous excitatory postsynaptic currents in dopaminergic neurons, which implies an endogenously activated tonic function for TRPV1 (Marinelli *et al.*, 2003). In addition, the involvement of TRPV1 in memory formation, anxiety, epilepsy, depressive behaviours, as well as in processes relevant for the mechanisms of Alzheimer and Parkinson diseases and schizophrenia, has been proposed (Fu *et al.*, 2009; You *et al.*, 2012). Additional TRP channels seem to contribute to some central nervous functions. TRPC6 channels promote dendritic growth and are selectively activated by hyperforin, the active principle of St. John's wort extract, which is responsible for the antidepressive profile of the herbal preparation (Leuner *et al.*, 2007). Through the same mechanisms, TRPC6 channels might also be involved in other brain disorders (Leuner *et al.*, 2007; Chahl, 2011). Thus, TRPA1 may be added to the list of TRP channels, which contribute to both physiological and pathological functions in the CNS.

Drugs increasing the synaptic availability of serotonin and norepinephrine have long been used for the treatment of depression. However, significant symptom improvement requires weeks of treatment, and a first course of therapy provides symptom relief to approximately 60% of patients. Therefore, innovative therapies are required for better management of mood disorders (for a review, see Berton and Nestler, 2006). Several ion channels have been proposed as potential therapeutic targets for the treatment of depression and anxiety. These include voltage-gated calcium (N-type), potassium (Kv7), serotonin 5-HT_3, purinergic P2X7 channels, and glutamate receptors, such as AMPA and NMDA (for a review, see Amiel and Mathew, 2007; Lodge and Li, 2008; Mico and Prieto, 2012). With the exception of pain signalling, current information regarding the role of TRPA1 in the brain is limited. In particular, little is known regarding the distribution and function of TRPA1 channels in discrete brain areas, thus preventing a specific localization of the overall behavioural effects observed in the present study. Nevertheless, our findings support the view that the TRPA1 channel could be a novel therapeutic target for the treatment of anxiety and mood disorders.

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Author contributions

E. A., E. C. G. and P. G. defined the experimental design of this study and wrote the final version of this manuscript. V. P. S. R. managed the literature searches and analyses. J. C. M. and M. M. undertook the experimental procedures (behavioural analysis) at Federal University of Rio Grande do Norte, Natal, Brazil. R. N. and S. M. undertook the experimental procedures (behavioural analysis in the elevated plus maze test and open field test) at University of Florence. I. M. M. and D. M. undertook the experimental procedures (behavioural analysis in the FST) at University of Florence. D. P. synthesized and purified the HC030031. B. L.-S. undertook the statistical analysis. J. C. M. wrote the first draft of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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