Evidence for the pathophysiological relevance of TRPA1 receptors in the cardiovascular system *in vivo*

Gabor Pozsgai[1], Jennifer V. Bodkin[1], Rabea Graepel[1], Stuart Bevan[2], David A. Andersson[2], and Susan D. Brain[1]*

[1]King's College London British Heart Foundation Centre, Cardiovascular Division and Centre of Integrative Biomedicine, King's College London, Franklin-Wilkins Building, London SE1 9NH, UK; and [2]Wolfson Centre for Age Related Diseases, Guy's Campus, London SE1 1UL, UK

Aims	The aim of the study is to investigate transient receptor potential ankyrin 1 (TRPA1)-induced responses in the vasculature and on blood pressure and heart rate (HR), in response to TRPA1 agonists using wild-type (WT) and TRPA1 knockout (KO) mice.
Methods and results	TRPA1 agonists allyl isothiocyanate and cinnamaldehyde (CA) significantly increased blood flow in the skin of anaesthetized WT, but not in TRPA1 KO mice. CA also induced TRPA1-dependent relaxation of mesenteric arteries. Intravenously injected CA induced a transient hypotensive response accompanied by decreased HR that was, depending on genotype and dose, followed by a more sustained dose-dependent pressor response (10–320 µmol/kg). CA (80 µmol/kg) induced a depressor response that was significantly less in TRPA1 KO mice, with minimal pressor effects. The pressor response of a higher CA dose (320 µmol/kg) was observed in WT but not in TRPA1 KO mice, indicating involvement of TRPA1. Experiments using TRP vanilloid 1 (TRPV1) KO and calcitonin gene-related peptide (CGRP) KO mice provided little evidence for the involvement of TRPV1 or CGRP, nor did blocking substance P receptors affect responses. However, the cholinergic antagonist atropine sulphate (5 mg/kg) significantly inhibited the depressor response and slowed HR with CA (80 µmol/kg), but had no effect on pressor responses. The pressor response remained unaffected, even in the presence of the ganglion blocker hexamethonium bromide (1 mg/kg). The α-adrenergic blocker prazosin hydrochloride (1 mg/kg) significantly inhibited both components, but not slowed HR.
Conclusion	TRPA1 is involved in mediating vasodilation. TRPA1 can also influence changes in blood pressure of possible relevance to autonomic system reflexes and potentially to vasovagal/neurocardiogenic syncope disorders.
Keywords	TRPA1 • CGRP blood flow • Blood pressure • Knockout mice

1. Introduction

The discovery of a novel superfamily of receptors, 'the transient receptor potential' (TRP) channels, which are non-selective cation channels, has allowed an increased understanding of mechanisms via which cells can be activated. The family includes TRP vanilloid 1 (TRPV1) and TRPA ankyrin 1 (TRPA1) in mammals.[1] The TRP channels play an important role in pain processing, but we are just beginning to unravel their complex role in the cardiovascular system.[2] The TRPA1 channel is expressed in unmyelinated and thinly myelinated

sensory neurons,[3] usually in a subset (approximately 50%) of TRPV1 expressing peptidergic neurons.[4–7] TRPA1 has been demonstrated on murine post-ganglionic sympathetic neurons,[8,9] human moto-neurons, neurons of the human intestinal myenteric plexus,[10] and non-neuronal tissues, including basal keratinocytes[10,11] and rat endothelial cells.[12]

TRPA1 can be activated by a range of substances, with knowledge to date mainly coming from *in vitro* studies. Cysteine residues in the N-terminus of the receptor can be modified reversibly and covalently by reactive electrophiles, leading to receptor activation.[13,14]

Exogenous agonists include the pungent plant extracts cinnamalde-hyde (CA) (*trans*-CA),[15] mustard oil (allyl isothiocyanate, AITC),[16] and garlic (allicin).[17] Endogenous agonists include products of oxi-dative stress/lipid peroxidation pathways, e.g. 4-hydroxynonenal and 4-oxononenal.[18,19]

The understanding of the cardiovascular regulatory and pathophy-siological potential of TRPA1 *in vivo* is limited. Topical administration of the TRPA1 agonist AITC to the mouse ear mediates vasodilatation owing to the release of neuropeptides, including calcitonin gene-related peptide (CGRP).[20] TRPA1 agonists have been shown to stimulate CGRP-dependent, cyclo-oxygenase-independent relax-ation of rat mesenteric arterial rings.[17] CA induces relaxation via endothelial-dependent (involving nitric oxide) and -independent mechanisms.[21] Rat cerebral arteries are relaxed by AITC acting via an endothelial-dependent mechanism that is associated with $K_{Ca}3.1$ channels, proposed to be a TRPA1-dependent mechanism.[12]

To our knowledge, no studies have been carried out to examine the peripheral influence of TRPA1 on blood flow and pressure *in vivo*. We demonstrate that TRPA1 agonists induce peripheral vaso-dilation. The effect of *trans*-CA on respiratory rate, heart rate (HR), and blood pressure has been known for some years.[22,23] We have, therefore, used this TRPA1 agonist, which unlike others can be suffi-ciently dissolved for intravenous administration, and examined the effect of CA-induced TRPA1 activation on HR, blood pressure, and local blood flow in the anaesthetized mouse.

2. Methods

2.1 Animals

Experiments were performed under the UK Animals (Scientific Pro-cedures) Act, 1986 and local KCL Ethics approval. The investigation con-forms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mice were maintained on normal diet in a climatically controlled environment. For genetically modified mice, male and female mice (25–35 g, >7 weeks old, age-matched), wild-type (WT) and TRPA1 knockout (KO) mice (C57BL/6-B6129P1/F2J mixed genetic background),[24] WT and TRPV1 KO (C57BL6/129SVJ strain),[25] or WT and CGRP KO (C57BL/6 strain) were used.[20] CD-1 mice (female, 25–30 g, Charles River, UK) were also used. All agents were from Sigma-Aldrich, UK, unless stated.

2.2 Measurement of skin blood flow

Skin blood flow was measured concomitantly in both ears or hind paws of mice anaesthetized with ketamine (75 mg/kg) and medetomidine (1 mg/kg) using a laser Doppler flow meter (Moor Instruments, UK) and a PowerLab data acquisition system (AD Instruments, UK). A probe, allowing blood flow to be measured precisely at one point in the ear (1 mm^2 and to 1–2 mm depth), was placed on each ear or hind paw, and a baseline reading was obtained. AITC (20 μL of 1% solution) was topically applied to the ipsilateral ear and vehicle (paraffin oil, 20 μL) to the contralateral ear. CA (1 μmol/50 μL intraplantar, i.pl.) was given to the ipsilateral paw and vehicle (<0.5% EtOH and <0.5% Tween-80 in Tyrode) to the contralateral paw. Blood flow readings were taken for 30 min and data collected as flux units, which are proportional to blood flow.[25] Blood flow was also assessed using a Full-Field Laser Perfusion Imager (Moor Instruments), which measures blood flow in a similar manner but using a mirror system to visualize the entire area of interest.[25]

2.3 Vascular relaxant activity

First-order mesenteric artery branches were isolated, mounted, and nor-malized on a wire myograph (DMT 610A) using 0.025 mm tungsten wire.

They were maintained in Krebs solution (118 mM NaCl, 24 mM NaHCO$_3$, 1 mM MgSO$_4$, 4 mM KCl, 0.5 mM NaH$_2$PO$_4$, glucose 5.5 mM, and CaCl$_2$ 2.5 mM), gassed with air/5% CO$_2$, and warmed to 37.4°C. Tissues were preconstricted with U46619 (10^{-8} M, Biomol, UK). Relaxation to CA (3–300 μM) or vehicle (ethanol) is expressed as percentage of precon-struction after 5 min. Endothelium was removed by gentle rubbing, and the presence of endothelium was assessed as >60% relaxation with car-bachol (10 μM).

2.4 Blood pressure and HR measurements

Mice were anaesthetized with urethane (2.5 g/kg), and a tracheal tube was inserted. The left carotid artery was cannulated and connected to a pressure transducer. The pressure line was filled with heparinized saline (100 U/mL 0.9% sodium chloride, pyrogen-free; Baxter Healthcare, UK, with heparin sodium, CP Pharmaceuticals, UK). Arterial pressure was monitored by a PowerLab data acquisition system (AD Instruments), and blood pressure and HR responses were determined. Drugs were injected into the right external jugular vein via a 30G needle. Temperature was maintained with a heating pad, and exposed tissues were moistened with saline. CA or vehicle (0.05% Tween-80, 0.2% ethanol) was given after 15 min equilibration for CD-1 mice or 60 min equilibration for TRPA1, TRPV1, and CGRP WT/KO mice. Other agents SR140333 (320 μg/kg), atropine sulphate (2.5 mg/mL, 5 mg/kg), prazosin hydrochlo-ride (0.5 mg/mL, 1 mg/kg), and hexamethonium bromide (0.5 mg/mL, 1 mg/kg) were dissolved in saline, injected 5 min before CA, and con-trolled internally.

2.5 Study design, data analysis, and statistics

Studies to determine the effect on local blood flow in the ear and paw and in mesentery were carried out in WT and TRPA1 KO mice (*Figure 1A*: $n = 1$ for each; *B*: $n = 6$ for each; *C*: WT $n = 7$, TRPA1 KO $n = 9$; and *D*: WT $n = 5$, $n = 10$ for others). The effect of CA on HR and blood pressure was examined first in CD-1 mice (*Figure 2* and *Table 1*, $n = 5$ for 10, 40, and 320 μmol/kg and $n = 6$ for control, 20, 80, and 160 μmol/kg CA) and then WT and TRPA1 KO mice (*Figure 3*, WT $n = 7$, TRPA1 KO $n = 5$ at 80 μmol/kg CA; $n = 6$ for depressor and $n = 4$ for pressor changes in WT mice at 320 μmol/kg CA; $n = 7$ for depressor and $n = 5$ for pressor changes in KO animals at 320 μmol/kg CA). The possible involvement of TRPV1 was evaluated using WT and TRPV1 KO mice (*Figure 4*, WT, $n = 5$ for each) and of the neuropeptides substance P and CGRP. This was tested using CGRP WT and KO mice ($n = 4$ for each) and through use of the selective NK$_1$ receptor antagonist SR140333, effective in the mouse *in vivo*.[25] (*Figure 5*, $n = 5$). The partici-pation of the parasympathetic vagal nerve was tested using the muscarinic receptor antagonist atropine sulphate ($n = 6$). The sympathetic involve-ment was investigated using the selective α$_1$ receptor blocker prazosin hydrochloride at the same dose as used by Oh-hashi *et al.*[26] to assess con-tribution of sympathetic tone in mice ($n = 5$). Finally, ganglionic blockade was examined with a combination of the nicotinic receptor antagonist hexamethonium bromide and atropine sulphate (5 mg/kg, similar to pre-vious publications, see *Figure 6*, $n = 6$).[26,27] Data are shown as mean $\pm$ SEM, and n indicates the number of animals used. Statistical analysis was by ANOVA + Tukey's, Dunnett's, or Bonferroni's *post hoc* test. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Effect of TRPA1 agonists on blood flow in the cutaneous microvasculature and vascular relaxation

AITC induced an increase in ear blood flow in WT mice, but not in TRPA1 KO mice that were sustained over the 30 min duration of
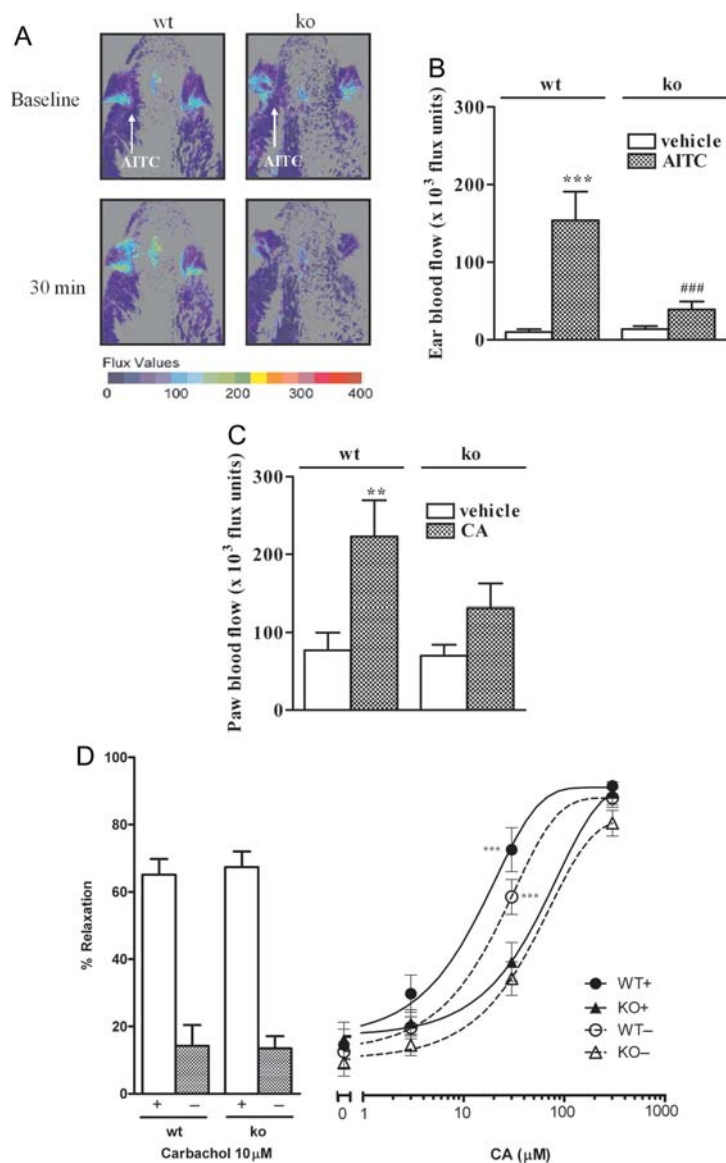
Figure 1 Effect of TRPA1 agonists on blood flow and vascular reactivity in WT and TRPA1 KO mice. (*A*) Representative image of blood flow using the FLPI scanner at baseline and 30 min following application of 20 μL AITC (1%) to the left ear and 20 μL of vehicle to the contralateral ear. (*B*) Blood flow ($\times 10^3$ flux units) measured over 30 min in the ears of WT and TRPA1 KO mice using laser Doppler probe. AITC (20 μL of 1%) was applied to one ear and 20 μL of vehicle to the contralateral ear. ***$P < 0.001$ vs. vehicle. ###$P < 0.001$ vs. AITC-treated ears of WT. (*C*) Blood flow ($\times 10^3$ flux units) over 30 min in the hind paws of WT and TRPA1 KO mice using laser Doppler probe. CA (1 μmol/50 μL i.pl.) injected into one hind paw and vehicle into contralateral paw. **$P < 0.01$ vs. vehicle-treated paws of WT. (*D*) CA-induced vascular relaxation was measured from preconstricted mesenteric arteries ± endothelial cells (WT+, KO+ with and WT−, KO− without endothelial cells) using a wire myograph. CA (3–300 μM) or vehicle (ethanol, 1.5 μL) or carbachol (10 μM) was used. ***$P < 0.001$ vs. respective KO response.

the experiment as determined via the laser Doppler scanner (*Figure 1A*) and probe (*Figure 1B*). *Trans*-CA (1 μmol/50 μL i.pl.) triggered a significant increase in the hind paw blood flow in WT, but not in TRPA1 KO mice (*Figure 1C*). CA-induced dose-dependent relaxation in WT and TRPA1 KO mesenteric arterial rings *in vitro*. This relaxation was significantly less potent in TRPA1 KO compared with WT arteries (*Figure 1D*), indicating a TRPA1-relaxant component. A reduced but significant response was observed in the absence of endothelium.

3.2 Effect of CA on mean arterial pressure and HR in CD-1 mice

CA injected into the external jugular vein induced a sudden and short-lasting fall in blood pressure, followed by a sustained elevation (*Figure 2*). The depressor phase involved a decrease in the mean arterial pressure (MAP) and HR. Baseline values, together with the peak change for the depressor and pressor results, are shown in *Table 1* for CD-1 mice. The depressor response was not dose-related; however, the pressor response exhibited a dose–response
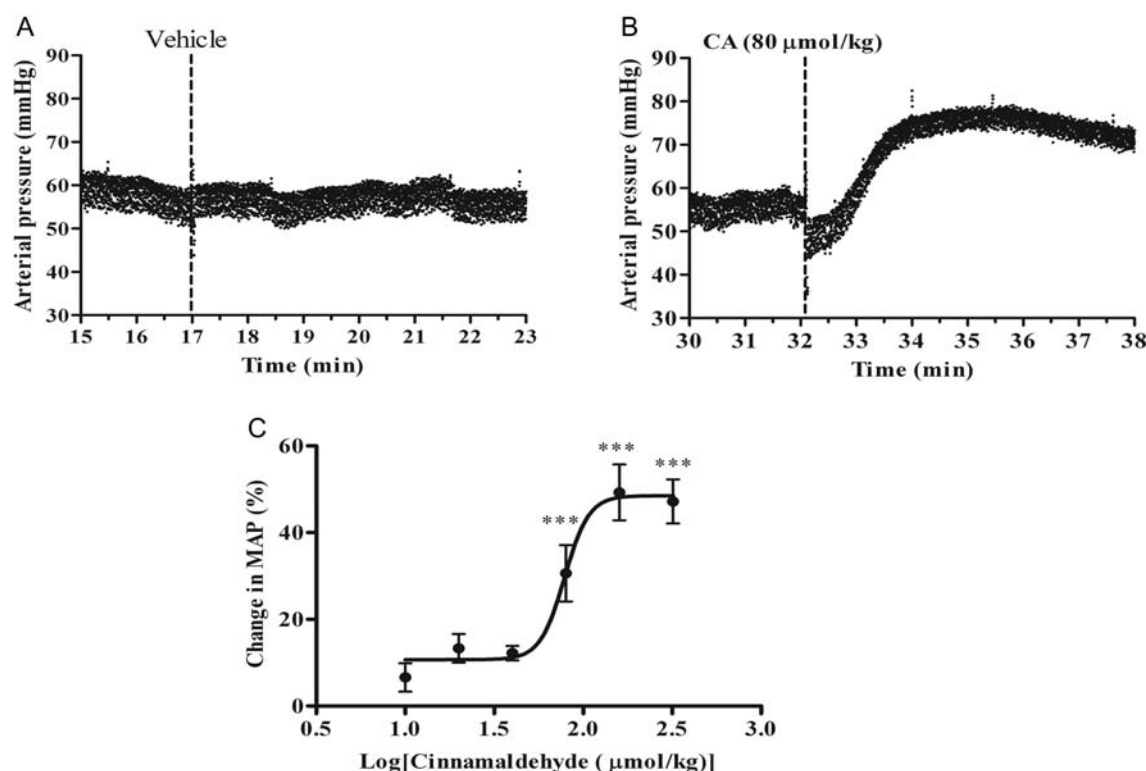
Figure 2 Effect of CA on blood pressure response in CD-1 mice. (A and B) Representative traces of arterial pressure for CA (80 μmol/kg, i.v.) and vehicle. CA evoked a rapid depressor and a sustained pressor reaction. (C) Dose–response relationship of pressor blood pressure changes induced by CA (i.v.), shown as per cent change in MAP compared with control (the highest dose of vehicle used). $ED_{50} = 78.56$ μmol/kg, ***$P < 0.001$ vs. control treatment.

relationship with an ED_{50} value of 78.6 μmol/kg (*Figure 2C*). In subsequent studies, 80 and 320 μmol/kg CA was used, with the higher dose used for some of the background strains, in order to investigate a significant pressor effect.

3.3 Blood pressure changes evoked by CA in WT and TRPA1 KO mice

Baseline MAP and HR for anaesthetized TRPA1 WT and KO mice were similar (WT: 65 ± 5 mmHg and 522.6 ± 19.5 b.p.m. and TRPA1 KO: 62.3 ± 4.4 mmHg and 531.6 ± 18 b.p.m.). CA (80 μmol/kg) evoked a significant hypotensive response in TRPA1 WT animals, which was accompanied by a decrease in HR (*Figure 3A* and *B*); however, a later pressor response was not observed to a significant degree in this strain at this dose. Of importance, TRPA1 KO mice exhibited significantly less hypotension and HR change in response to 80 μmol/kg CA. The dose of CA was increased to 320 μmol/kg, in order to investigate a pressor response in this strain, and a typical biphasic response, consisting of a transient hypotension, followed by hypertension as previously observed in CD-1 mice, was observed (*Figure 3C* and *D*). The hypotensive responses to this higher dose were similar in both WT and KO mice. However, the pressor response was observed in WT but not in TRPA1 KO mice, indicating that TRPA1 plays an important role in this component of the response. Thus these results, at two different doses, indicate a potential involvement of TRPA1 in both depressor and pressor responses.

3.4 Role of TRPV1 and the major dilator peptides CGRP and substance P on the depressor action of CA

As TRPA1 is co-expressed with TRPV1 on peptidergic sensory fibres, we examined the possible participation of TRPV1, using TRPV1 WT and KO mice. Baseline MAP and HR levels of WT and KO animals were 49.8 ± 2.1 mmHg and 543.1 ± 18.3 b.p.m. and 52 ± 2.4 mmHg and 482.4 ± 16.6 b.p.m., respectively. CA (80 μmol/kg) induced small, similar changes in TRPV1 WT and KO mice (*Figure 4A* and *B*). The higher dose of 320 μmol/kg CA induced greater changes (*Figure 4C* and *D*). The only significant difference observed was a higher pressor response in TRPV1 KO mice, compared with WT mice. These results indicate that TRPV1 receptors do not mediate the pressor response to CA. However, it is possible that the major vasoactive neuropeptides CGRP and substance P may play a role, through a selective TRPA1 activating mechanism. The contribution of CGRP was tested using CGRP WT and KO animals. Baseline MAP and HR data for WT and KO mice were 54.4 ± 2.9 mmHg and 431.5 ± 28.9 b.p.m. and 57.8 ± 2.5 mmHg and 467.9 ± 11.1 b.p.m., respectively. WT and CGRP KOs exhibited similar hypotensive effects in response to CA, suggesting a lack of involvement of CGRP. In addition, other components of the response were similar in WT and CGRP KO mice (*Figure 5A* and *B*).

The possible involvement of substance P was evaluated through use of SR140333, an effective NK_1 receptor antagonist in mouse *in vivo*.[25]

Table 1 Effect of different doses of CA on MAP and HR of CD-1 mice

	Baseline		Depressor		Pressor	
Dose of CA (μmol/kg)	MAP (mmHg)	HR (b.p.m.)	Change in MAP (%)	Change in HR (%)	Change in MAP (%)	Change in HR (%)
Control	52.1 ± 1.7	426.6 ± 37.6	−1.5 ± 2.2	−2.8 ± 1.3	−1.5 ± 2.2	−2.8 ± 1.3
10	56.8 ± 1.7	511.6 ± 30.2	0.6 ± 1.4	−1.6 ± 0.5	4.2 ± 2.9	2.5 ± 1.2
20	54.5 ± 1.7	442.3 ± 33.3	−6.9 ± 6.2	−6.6 ± 3.8	10.2 ± 0.7	1.1 ± 2.3
40	56.4 ± 0.6	559.5 ± 47.4	−19.2 ± 4.5[a]	30.3 ± 8.7[b]	11.8 ± 2.1	−3.3 ± 3.3
80	58 ± 2.0	495.9 ± 21.4	−23 ± 6.9[b]	29.3 ± 9.2[b]	25.7 ± 8.9[b]	−6.9 ± 3.2
160	52.4 ± 2.2	457.6 ± 36.1	−17.4 ± 5.1[c]	−20.6 ± 4.4	55.9 ± 7.6[b]	11.5 ± 8.5
320	55.1 ± 2.6	483.6 ± 39.4	−6.5 ± 0.8	−22.7 ± 3.4[c]	53.9 ± 5.3[b]	29.1 ± 29.9[b]

Different doses of CA were injected i.v. into CD-1 animals. Arterial pressure was monitored. Per cent changes in MAP and HR are shown in response to doses of CA as seen during depressor and pressor reactions. This table includes baseline MAP and HR values for each animal group. Control represents vehicle of the highest dose of CA. $n = 5–6$. Data are presented as mean ± SEM.
[a]$P < 0.01$ vs. vehicle treatment.
[b]$P < 0.001$ vs. vehicle treatment.
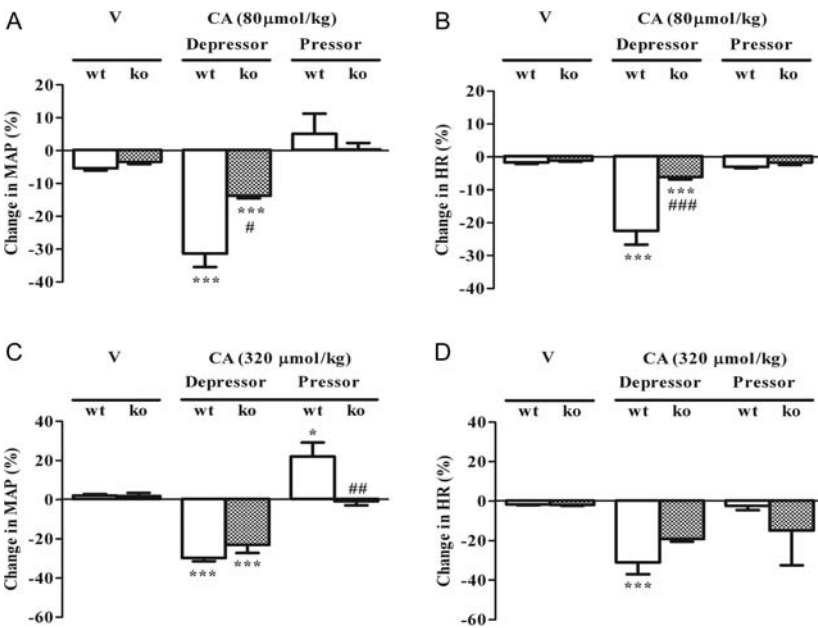[c]$P < 0.05$ vs. vehicle treatment.

Figure 3 Effect of CA on MAP and HR in TRPA1 WT and TRPA1 KO mice. Per cent changes of (*A*) MAP and (*B*) HR in response to 80 μmol/kg CA i.v. showing vehicle treatment (V) and depressor and pressor reactions. Changes of (*C*) MAP and (*D*) HR in response to 320 μmol/kg CA i.v. *$P < 0.05$, ***$P < 0.001$ vs. vehicle of CA (V). #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ vs. WT.

Baseline values for MAP and HR were 50.4 ± 2.1 mmHg and 519.6 ± 16.4 b.p.m. The antagonist (320 μg/kg) did not influence the depressor response induced by CA (80 μmol/kg) in CD-1 mice (*Figure 5C* and *D*), suggesting a lack of involvement of the NK₁ receptor.

3.5 Effect of pharmacological blockers of autonomic nerves on the biphasic response to CA

Atropine sulphate (5 mg/kg) significantly inhibited the hypotensive and HR changes involved in the depressor response in CD-1 mice.

Atropine had no effect on the pressor component (*Figure 6A* and *B*). Basal values were 52.9 ± 1.6 mmHg for MAP and 488 ± 27.8 b.p.m. for HR. Prazosin hydrochloride (1 mg/kg) also significantly inhibited the fall in blood pressure. The HR data showed a similar trend, although prazosin did not have a significant effect. A significantly lower pressor response was present in prazosin-treated mice compared with control mice (*Figure 6C* and *D*). Basal values for MAP and HR were 51.4 ± 3.2 mmHg and 466.3 ± 43.7 b.p.m., respectively. Ganglionic blockade was achieved by injecting atropine sulphate (5 mg/kg) and hexamethonium bromide (1 mg/kg). This did not influence the pressor response, and as in the previous set of
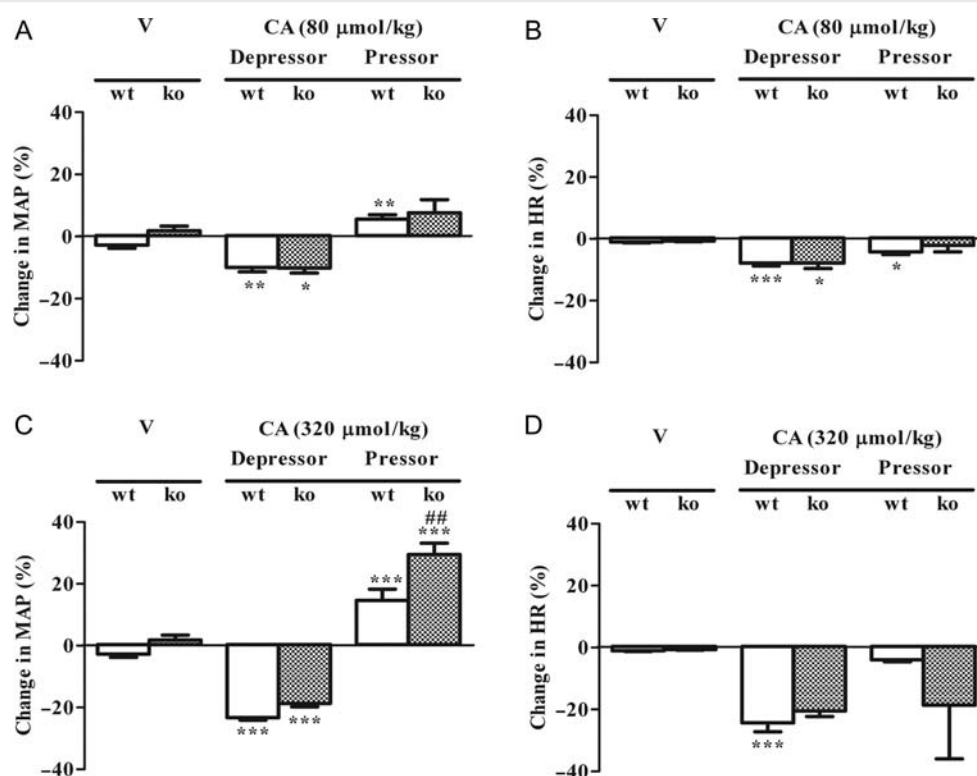
Figure 4 Effect of CA (80 μmol/kg, i.v.) on (*A*) MAP and (*B*) HR in TRPV1 WT and TRPV1 KO mice. Effect of CA (320 μmol/kg, i.v.) on (*C*) MAP and (*D*) HR in WT and TRPV1 KO mice. Results shown as per cent change in MAP and HR. Responses to vehicle (V) and depressor/pressor phases induced by CA are shown. ***$P < 0.001$ vs. vehicle of CA (V). ##$P < 0.01$ vs. WT.

experiments, the depressor and HR increases were significantly inhibited by atropine (*Figure 6E* and *F*). Baseline values were 53 ± 1.5 mmHg and 485.6 ± 32.7 b.p.m. for MAP and HR, respectively.

4. Discussion

We show that the TRPA1 agonist AITC, which unlike CA can be applied topically, increases peripheral blood flow in WT but not in TRPA1 KO mice. Similarly, i.pl. injections of CA stimulate a significant increase in paw blood flow in WT but not in TRPA1 KO mice and relaxed mesenteric vessels from WT mice in a more potent manner than those from TRPA1 KO mice. These results indicate that TRPA1 activation, in addition to TRPV1 activation, mediates increased blood flow and vascular relaxation. The results imply that activation by endogenous TRPA1 agonists will lead to increased local peripheral blood flow. Previously, before the realization that AITC is a TRPA1 agonist, we demonstrated that AITC-induced blood flow is mediated by substance P and CGRP in the mouse ear.[20] We therefore suggest that activation of TRPA1 agonists on peripheral nerves leads to local sensory neurogenic vasodilatation. In contrast, antagonists of the major vasoactive receptors for substance P and CGRP have now been used clinically, and no marked effects on baseline blood pressure have been observed.[28,29] TRPA1-mediated neurogenic vasodilation involving neuropeptides is therefore unlikely to regulate blood pressure under physiological conditions. However, we now present major new evidence that activation of TRPA1 induces acute reflex changes in blood pressure and HR in

the anaesthetized mouse. We provide evidence that vasovagal responses are implicated in the common neurocardiogenic syncope. Furthermore, results suggest that substance P and CGRP do not contribute to the decrease in blood pressure evoked by systemic TRPA1 activation.

There are early observations on the cardiovascular effects of agents that are now considered to exhibit TRPA1 agonist activity (e.g. CA, 2-chlorobenzilidene malononitrile and dibenz[b,f][1,4]oxazepine). The results range from general observations relevant to CA mediating cardiovascular effects including either depressor or pressor effects[23,30,31] to a more precise mechanistic study in which the pressor response to CA could be antagonized by the α-adrenergic blocker.[22] Here using the mouse for the first time, we suggest that the depressor response represents central reflexes following TRPA1 activation. The depressor response and a decrease in HR observed in response to the lower dose of CA were significantly blunted in TRPA1 KO mice, as was the pressor response observed in response to a high dose of CA (320 μmol/kg) and also the relaxant response in mesenteric arteries *in vitro*. Evidence for a TRPA1-dependent relaxant component in mesenteric arteries was obtained as responses were significantly reduced to CA (30 μM) in tissues from TRPA1 KO mice. The relaxant response in WT mice was composed of a small endothelial-dependent and a clear independent component, in contrast to a study in rat cerebral arteries,[12] but in support of previous findings from Yanaga *et al.*[21] for CA in the rat aorta. In addition, Bautista *et al.*[17] have shown relaxation of AITC in rat mesenteric arteries, via a TRPV1-independent mechanism that involved
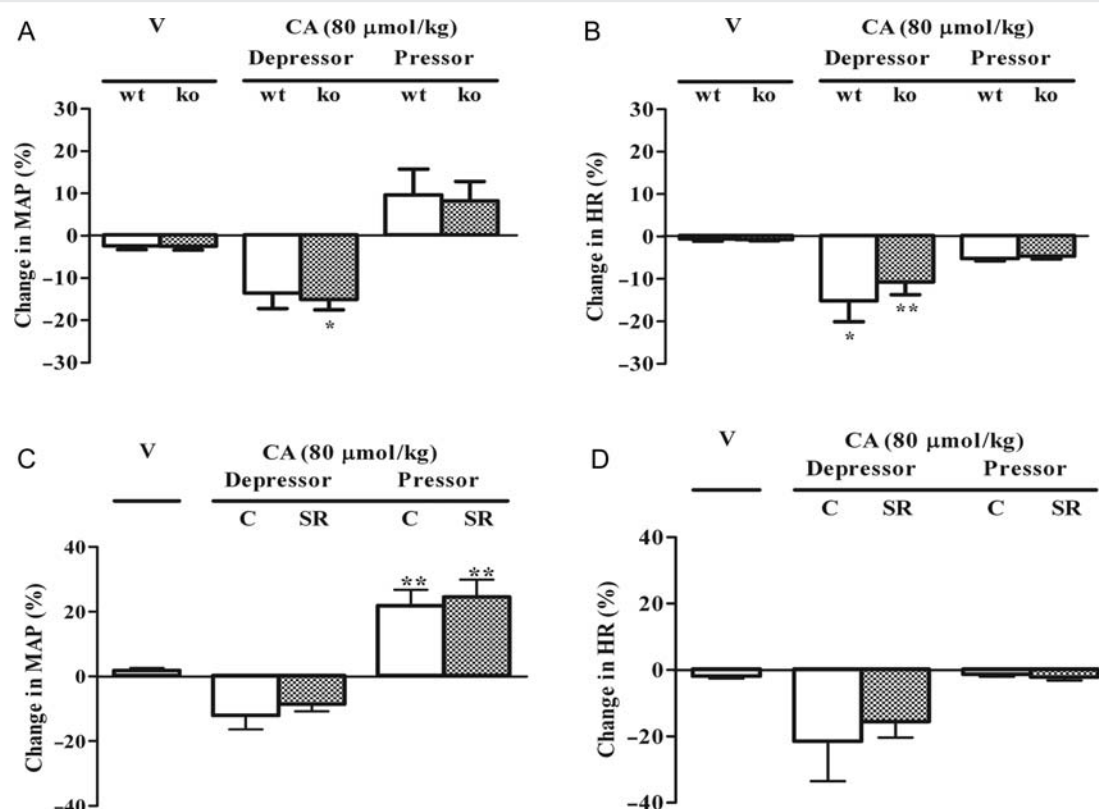
Figure 5 Effect of CA (80 μmol/kg, i.v.) on (*A*) MAP and (*B*) HR of WT and CGRPKO mice. Results shown as per cent changes in MAP and HR to vehicle (V) and to CA during depressor/pressor reactions evoked by *trans*-CA. $n = 4$. Data are presented as mean $\pm$ SEM. *$P < 0.05$ and **$P < 0.01$ vs. vehicle of CA (V). Effect of NK$_1$ receptor antagonist SR140333 (SR, 320 μg/kg, i.v.) on *trans*-CA-induced (80 μmol/kg, i.v.) cardiovascular changes in CD-1 mice. Arterial pressure was recorded. Percentage changes in (*A*) MAP and (*B*) HR were plotted. The figure shows response to control and depressor/pressor phenomena. Vehicle of CA (V) and solvent of SR140333 (C) were used as controls. **$P < 0.01$ vs. vehicle of CA (V).

CGRP. TRPA1 involvement was not directly investigated in the latter two studies. We conclude that *in vitro* vascular studies to date indicate that TRPA1 may act via different vascular mechanisms depending on location and tissue.

Based on the knowledge that TRPA1 is expressed on a subpopulation of TRPV1 expressing peptidergic neurons in the vagus,[4,32] we next investigated participation of TRPV1 and CGRP and SP in depressor changes induced by CA. WT and TRPV1 KO mice exhibited similar depressor and pressor phenomena, except that the higher dose of CA (320 μmol/kg) induced significantly higher blood pressures in the pressor phase in TRPV1 KO mice when compared with WT mice. This suggests that a functional interaction may exist *in vivo* between TRPA1 and TRPV1, which leads to a vasodilator response. Interactions between TRPV1 and TRPA1 have been reported in isolated neurons and in peripheral tissues,[15,33–35] although mechanisms involved are unclear. The most obvious interaction is that the two channels, co-localized to sensory nerves, influence the release and participation of sensory nerve-derived vasodilator neuropeptides CGRP and substance P in an interactive manner. However, results, where the CGRP and substance P components were deleted, indicate that the sensory nerve-derived vasodilators are unlikely to be involved in the observed systemic responses to CA.

Both historical and recent *in vivo* studies involving inhaled or i.v. CA have demonstrated a slowing of respiratory rate,[23,32] probably due to pulmonary chemoreflex activation by CA.[32] Some early *in vivo* studies

on TRPA1 agonists demonstrated bradycardia accompanying bradypnoea.[23,30] Our own findings, together with these previous results, suggest that systemic or pulmonary TRPA1 activation stimulates the Bezold–Jarisch reflex. Given that the vagal fibres innervating the heart are similar to those supplying the lungs, we questioned what effect TRPA1 activation may have on cardiovascular parameters. The role of the effector branches of vasovagal reflexes in CA-evoked blood pressure changes was analysed. The sudden and short depressor changes in blood pressure and also the decrease in the pulse rate were significantly alleviated by both atropine and prazosin pretreatment. This indicates a classic vasovagal reflex activation with an increase in parasympathetic vagal output to the heart, associated with lowered HR and blood pressure, and decreased sympathetic activity on resistance vessels, associated with decreased peripheral resistance and hypotension.[36,37] In contrast, the pressor blood pressure changes were significantly inhibited by α_1 receptor blockade, but were unaffected by ganglionic blockade. These data provide evidence that the increase in blood pressure is mediated by peripheral sympathetic activation and noradrenaline release. Of note, both TRPV1 and TRPA1 receptor agonists have been shown to induce capsaicin-depletion sensitive adrenaline secretion from the adrenal gland.[38,39] In our studies, ganglionic blockade had no effect on hypertensive responses to CA; therefore, we assume that pressor changes were due to the activation of sympathetic fibres distal from ganglia. In support of this possibility, studies on cold sensitive sympathetic
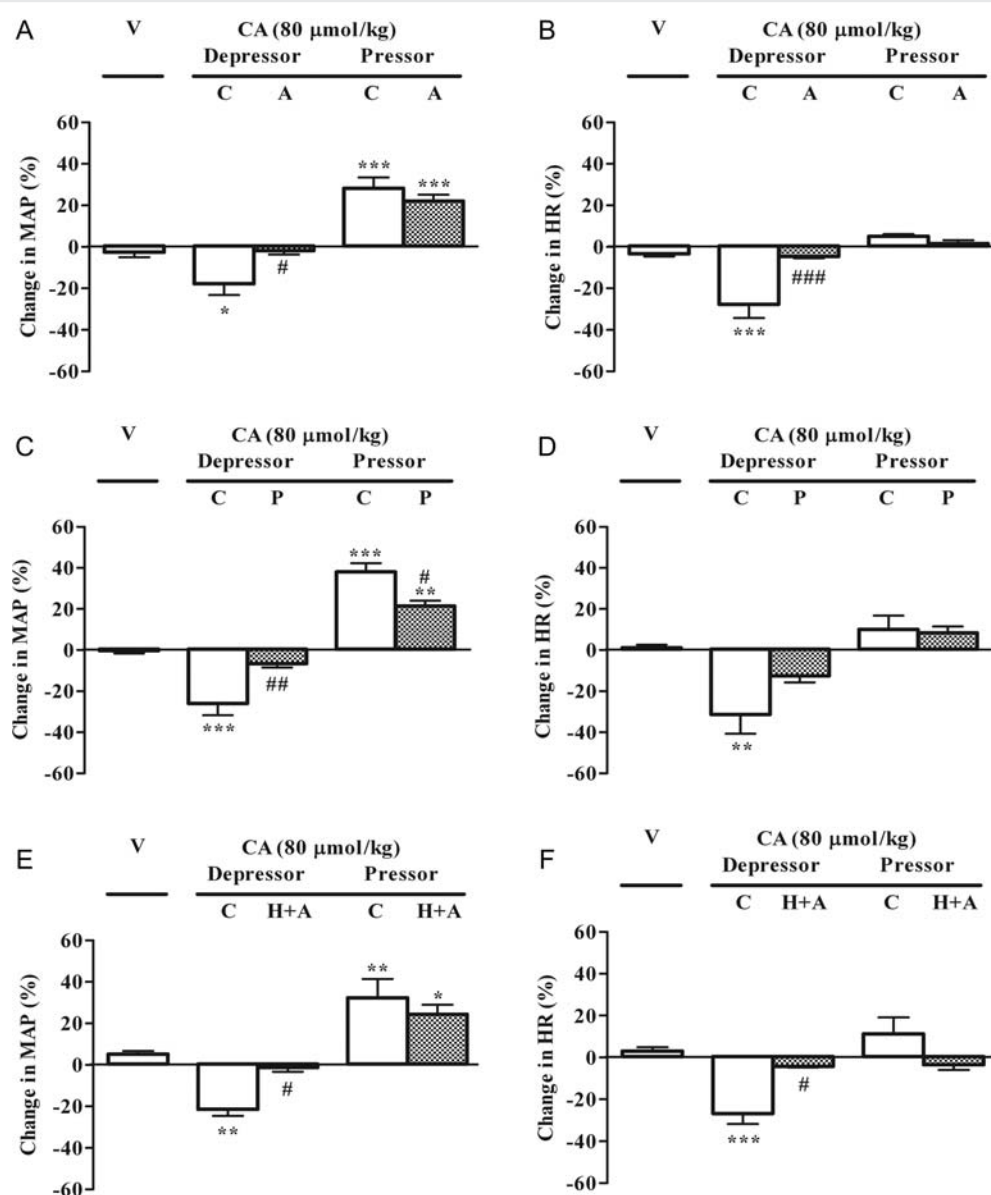
Figure 6 Effect of muscarinic acetylcholine, adrenergic α_1 receptor, and ganglionic blockade on *trans*-CA-induced (80 μmol/kg, i.v.) cardiovascular changes in CD-1 mice. Arterial pressure was recorded. Percentage changes in MAP and HR were plotted. The figure shows responses to control and depressor/pressor phenomena. Vehicles of CA (V) and of atropine sulphate, prazosin hydrochloride, and hexamethonium bromide plus atropine sulphate (C) were used as controls. CA-induced (A) MAP and (B) HR changes after treatment with atropine sulphate (A, 5 mg/kg, i.v.). *Trans*-CA-evoked changes in (C) MAP and (D) HR after treatment with prazosin hydrochloride (P, 1 mg/kg, i.v.). Changes in (E) MAP and (F) HR in response to CA after treatment with hexamethonium bromide (5 mg/kg, i.v.) plus atropine sulphate (H + A, 5 mg/kg, i.v.). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ vs. vehicle of CA (V). #$P < 0.05$, ##$P < 0.01$, and ###$P < 0.001$ vs. vehicle control of respective drug (C).

elements report the presence of TRPA1 mRNA, but not TRPV1 or TRPM8, in murine superior cervical ganglia.[8,9] Thus we suggest that activation of post-ganglionic sympathetic fibres by CA results in vaso-constriction and a hypertensive reaction. These results provide evidence of the Bezold–Jarisch-like reflex. Activation of stretch sensing mechanoreceptors and chemoreceptors at various sites (involving cardiopulmonary afferents) initiate the reflex.[36,37] We do not know the location of the TRPA1 channels that initiate this reflex; however, the present evidence does suggest that activation is unlikely to involve TRPV1 receptors or neuropeptides. Possibly, vascular

endothelial cells that mediate TRPA1 relaxation in the rat cerebral arteries may be involved in the initiation of the response.[12] However, here we show that CA-mediated vascular relaxation involves a TRPA1-dependent but endothelial-independent response in mouse mesenteric arteries. In contrast, novel results suggest that TRPA1 may also be activated in the nucleus tractus solitarii,[40] which plays a central role in Bezold–Jarisch reflex.[41] It is unknown whether i.v. injected CA can directly activate these receptors.

To conclude, studies on the potential of TRPA1 to modulate cardiovascular responses are at an early stage. TRPA1-dependent

responses are indicated, although it is not possible to predict how the results using small numbers of mice will translate to humans at this stage. Although limitations exist in terms of a lack of selective ligands for TRPA1, the use of TRPA1 KO mice may be associated with compensatory mechanisms. However, it is noted that the TRPA1 KO mice used here have been used successfully by other groups.[24] We confirm that TRPA1 can act in a distinct manner in the periphery to mediate vasodilator responses, which previous evidence suggests is neuropeptide-dependent. However, evidence from TRPA1 KO mice and through the pharmacogenetic studies performed here suggests that autonomic neuronal pathways, rather than sensory neuropeptide pathways, are more important in the body's response to i.v. administration of the TRPA1 agonist CA, in the mouse at least. We provide evidence that suggests that the TRPA1 channel should be investigated for possible involvement in the Bezold–Jarisch reflex response that includes vasovagal reflexes, which are components of neurocardiogenic syncope. Although our studies have involved the use of an exogenous agonist, it should be noted that the high number of proposed endogenous agonists, especially those associated with oxidative stress, suggests an important focus for further studies involving understanding of the neurocardiogenic syncope.

Acknowledgements

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