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REVIEW

TRPA1 channels in the vasculature

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This review is focused on the role of the ankyrin (A) transient receptor potential (TRP) channel TRPA1 in vascular regulation. TRPA1 is activated by environmental irritants, pungent compounds found in foods such as garlic, mustard and cinnamon, as well as metabolites produced during oxidative stress. The structure of the channel is distinguished by the ~14–19 ankyrin repeat (AR) domains present in the intracellular amino terminus. TRPA1 has a large unitary conductance (98 pS) and slight selectivity for Ca^{2+} versus Na^{+} ions ($P_{Ca}/P_{Na} \approx 7.9$). TRPA1 is involved in numerous important physiological processes, including nociception, mechanotransduction, and thermal and oxygen sensing. TRPA1 agonists cause arterial dilation through two distinctive pathways. TRPA1 channels present in perivascular nerves mediate vasodilatation of peripheral arteries in response to chemical agonists through a mechanism requiring release of calcitonin gene-related peptide. In the cerebral circulation, TRPA1 channels are present in the endothelium, concentrated within myoendothelial junction sites. Activation of TRPA1 channels in this vascular bed causes endothelium-dependent smooth muscle cell hyperpolarization and vasodilatation that requires the activity of small and intermediate conductance Ca^{2+}-activated K^{+} channels. Systemic administration of TRPA1 agonists causes transient depressor responses, followed by sustained increases in heart rate and blood pressure that may result from elevated sympathetic nervous activity. These findings indicate that TRPA1 activity influences vascular function, but the precise role and significance of the channel in the cardiovascular system remains to be determined.

Abbreviations

15d-PGJ(2), 15-deoxy-delta(12,14)-prostaglandin J(2); 4-HHE, 4-hydroxyhexenal; 4-HNE, 4-hydroxy-2-nonenal; 4-ONE, 4-oxo-nonenal; AITC, allyl isothiocyanate; AR, ankyrin repeat; CA, cinnamaldehyde; CGRP, calcitonin gene-related peptide; DADS, diallyl disulfide; ER, endoplasmic reticulum; HR, heart rate; IP_3R, inositol trisphosphate receptor; $K_{Ca}2.3$, small conductance Ca^{2+}-activated K^{+} channel; $K_{Ca}3.1$, intermediate conductance Ca^{2+}-activated K^{+} channel; K_{IR}, inwardly-rectifying K^{+} channel; MAP, mean arterial pressure; MEJ, myoendothelial junction; N, amino terminus; NR, not reported; RSNA, renal sympathetic nerve activity; TRP, transient receptor potential

Introduction: a family of one

The mammalian transient receptor potential (TRP) superfamily of cation channels comprises 28 members assigned to six subfamilies based on sequence homology. The ankyrin (A) subfamily is the smallest and is composed of only a single member, TRPA1 (originally designated as ANKTM1). Despite being one of the last TRP channels to be discovered (Story *et al.*, 2003), TRPA1 has garnered a great deal of recent attention. The essential properties and structure of TRPA1 have been evolutionarily conserved for more than 500 million years (Kang *et al.*, 2010). The channel likely evolved as a sensor of electrophilic toxicity (Kang *et al.*, 2010) before divergent specialized functions developed in different species (Story *et al.*, 2003; Rosenzweig *et al.*, 2005; Cordero-Morales *et al.*, 2011; Geng *et al.*, 2011). TRPA1, like all TRP channels, is expressed as six-transmembrane domain polypeptide subunits, a motif common to many types of ion channels. Functional TRPA1 channels are formed from four of these subunits. Assembled TRPA1 channels are thought to have a homomeric structure (composed of four identical subunits), as there is currently no evidence that heteromultimeric

channels involving other TRP channel subunits can form. The channel is distinguished structurally by, and named for, the ~14–19 ankyrin repeat (AR) domains forming a portion of the protein's intracellular N-terminus. In general, AR domains mediate protein–protein interactions and provide mechanical elasticity (Sedgwick and Smerdon, 1999), although a recent study suggests that particular TRPA1 AR domains can regulate agonist- and heat-induced channel activity (Cordero-Morales *et al.*, 2011). TRPA1 was originally described as a non-selective cation channel that is equally permeable to Na^+ versus Ca^{2+} ions (P_{Ca}/P_{Na} reported as 0.84–3.28) (Story *et al.*, 2003; Wang *et al.*, 2008), although Karashima *et al.* found that during agonist stimulation, $P_{Ca}/P_{Na} = 7.91 \pm 0.60$ and the fractional Ca^{2+} current under these conditions is 17.9–22.3% (Karashima *et al.*, 2010). The unitary conductance of the channel is large (98 pS, when physiological ionic gradients are maintained) (Nagata *et al.*, 2005), indicating that TRPA1 channels can support consequential levels of Ca^{2+} influx. Predictably, TRPA1 has been shown to influence a broad range of physiological processes that involve Ca^{2+}-dependent signalling pathways, including nociception, mechanotransduction, thermal and oxygen sensing, and responses to environmental irritants and pungent compounds. This manuscript focuses on the role of TRPA1 channels in vascular regulation. The relevant pharmacology is discussed, and studies investigating the consequences of TRPA1 activity on local and integrative control of the vasculature are reviewed.

TRPA1 pharmacology: activators

TRPA1 channels are activated by a large, and still growing, list of diverse compounds, including acrolein (Bautista *et al.*, 2006), diesel exhaust (Hazari *et al.*, 2011), local anaesthetics (Leffler *et al.*, 2011), the non-steroidal anti-inflammatory analgesic acetaminophen (paracetamol) (Nassini *et al.*, 2010; Andersson *et al.*, 2011) and hydrogen sulfide (Streng *et al.*, 2008; Krueger *et al.*, 2010; Miyamoto *et al.*, 2011). In addition to these chemical stimuli, TRPA1 channels in various species are activated by physical factors, such as heat (Rosenzweig *et al.*, 2005; Cordero-Morales *et al.*, 2011), cold (Story *et al.*, 2003) and mechanical stress (Corey *et al.*, 2004; Kwan *et al.*, 2006; Vilceanu and Stucky, 2010). The role of TRPA1 channels in the sensation of noxious cold, recently reviewed in detail (Caspani and Heppenstall, 2009), remains controversial, with approximately equal numbers of studies for and against. For the purposes of the current review, TRPA1 activators present in plants used for food or traditional medicine, and those related to oxidative stress, are discussed in more detail because of their demonstrated or potential roles in vascular regulation.

Pungent dietary molecules

TRPA1 is activated by many substances found in commonly consumed foods and plants used in traditional medical practices of several cultures (Table 1). Allyl isothiocyanate (AITC) (Bandell *et al.*, 2004; Jordt *et al.*, 2004), allicin (Bautista *et al.*, 2005; Macpherson *et al.*, 2005) and cinnamaldehyde (Bandell *et al.*, 2004) (CA), derived from mustard oil, garlic and cinnamon, respectively, are the most commonly used TRPA1 activators. Although AITC can undoubtedly activate TRPA1 channels, recent studies suggest that AITC may have effects that are independent of TRPA1 (Capasso *et al.*, 2012; Everaerts *et al.*, 2011), highlighting the importance of selective pharmacology and/or TRPA1 knockout mice to demonstrate response specificity.

Activation of TRPA1 by AITC and other electrophilic compounds occurs through a unique mechanism involving covalent modification of cysteine residues present in the intracellular amino (N) terminus. Two studies examining specific residues required for channel activation identified five distinct cysteine potentially involved in covalent modification (Table 2), but only one (C622 in the mouse gene, corresponding to C619 in the human sequence) was identified by both groups (Hinman *et al.*, 2006; Macpherson *et al.*, 2007a).

Table 1

TRPA1 activators derived from pungent foods and traditional medicines

Agonist	Source	EC_{50}	References
Allyl isothiocyanate (AITC)	Mustard	11 ± 1 μM (rat) 33 μM (mouse)	Bandell *et al.* (2004); Jordt *et al.* (2004)
Allicin	Garlic	1.3 μM (mouse) 1.9 μM (human)	Bautista *et al.* (2005); Macpherson *et al.* (2005)
Cinnamaldehyde (CA)	Cinnamon	100 μM (mouse)	Bandell *et al.* (2004)
Eugenol	Cloves	NR	Bandell *et al.* (2004)
Gingerol	Ginger	NR	Bandell *et al.* (2004)
Nicotine	Tobacco	~10 μM (mouse)	Talavera *et al.* (2009)
Umbellulone	*Umbellularia californica* (headache tree)	56.6 ± 8.3 μM (rat)	Nassini *et al.* (2012); Zhong *et al.* (2011a)
Ligustilide	Celery, lovage, *Angelica sinensis, Ligusticum chuanxiong, Ligusticum porteri*	44 μM (mouse)	Zhong *et al.* (2011b)

NR, not reported.

Table 2

Amino acid residues and ankyrin repeat domains involved in TRPA1 activation

Species	Stimulus	Residue/domain	References
Mouse	AITC	C415	Macpherson *et al.* (2007a)
	AITC	C422	Macpherson *et al.* (2007a)
	AITC	C622	Macpherson *et al.* (2007a)
Human	AITC	C619	Hinman *et al.* (2006)
	AITC	C639	Hinman *et al.* (2006)
	AITC	C663	Hinman *et al.* (2006)
	AITC	K708	Hinman *et al.* (2006)
	Hyperoxia and DADS	C633	Takahashi *et al.* (2011)
	Hyperoxia and DADS	C856	Takahashi *et al.* (2011)
	Hypoxia	P394	Takahashi *et al.* (2011)
	AITC	AR 11–16	Cordero-Morales *et al.* (2011)
Crotalus atrox	Heat	AR 3–8; AR 10–15	Cordero-Morales *et al.* (2011)

Table 3

Activators of TRPA1 related to oxidative stress

Compound	EC_{50}	Reference
4-hydroxy-2-nonenal (4-HNE)	10–27 μM	Trevisani *et al.* (2007); Macpherson *et al.* (2007b); Andersson *et al.* (2008); Taylor-Clark *et al.* (2008)
4-oxo-nonenal (4-ONE)	1.5–1.9 μM	Andersson *et al.* (2008); Taylor-Clark *et al.* (2008)
4-hydroxyhexenal (4-HHE)	39–50 μM	Andersson *et al.* (2008); Taylor-Clark *et al.* (2008)
Hydrogen peroxide (H_2O_2)	230 μM	Andersson *et al.* (2008)
15-deoxy-delta(12,14)-prostaglandin J(2) [15d-PGJ(2)]	5.6 μM	Andersson *et al.* (2008)

Cysteine residues at C633 and C856 were shown to participate in activation of the human *TRPA1* gene by diallyl disulfide (DADS) (Takahashi *et al.*, 2011). In addition to these cysteine residues, a lysine residue (K708 in the human sequence) also contributes to AITC-induced activation of TRPA1 (Hinman *et al.*, 2006). Specific N-terminal AR domains are also involved in the regulation of TRPA1 activity, as demonstrated by an elegant study from the Julius lab using chimeric genes composed of *Crotalus atrox* (Western diamondback rattlesnake) and human TRPA1 sequences. ARs in the N-terminus of the *C. atrox TRPA1* gene (AR3-8 and AR10-15) involved in sensation of heat were identified (Cordero-Morales *et al.*, 2011). In contrast, AR domains in the human gene (AR11-16) were shown to be involved in sensitivity to AITC (Cordero-Morales *et al.*, 2011). Specific amino acid residues and AR domains involved in TRPA1 activation are summarized in Table 2.

Products of oxidative stress

A number of recent studies indicate that TRPA1 channels are activated by substances produced at high levels during oxidative stress, such as H_2O_2 and specific metabolites of lipid peroxidation (Table 3). Macpherson *et al.* (2007b) and Trevisani *et al.* (2007) first reported that TRPA1 channels are activated by 4-hydroxy-2-nonenal (4-HNE), an unsaturated aldehyde produced by lipid peroxidation at high levels during oxidative stress. Two similar substances generated during oxidative injury, 4-oxononenal (4-ONE) and 4-hydroxyhexenal (4-HHE), also stimulate TRPA1 channels (Taylor-Clark *et al.*, 2008). 4-ONE is nearly 10-fold more potent than 4-HNE, whereas 4-HHE is approximately fivefold less potent (Taylor-Clark *et al.*, 2008). A study by Andersson *et al.* systematically investigated the ability of several oxidative stress-related substances to activate TRPA1 and found that in addition to 4-HNE, 4-ONE and 4-HHE, TRPA1 channels are activated by H_2O_2 as well as the cyclopentenone prostaglandin 15-deoxy-delta(12,14)-prostaglandin J(2) [15d-PGJ(2)] (Andersson *et al.*, 2008). These findings strongly suggest that oxidative stress metabolites are endogenous activators of TRPA1 channels in mammalian systems, but the physiological significance of this response is uncertain.

In addition to sensing substances produced during oxidative stress, a recent study indicates that TRPA1 channels directly detect molecular oxygen and become active during

hyperoxic and hypoxic conditions through distinct molecular pathways (Takahashi *et al.*, 2011). This report demonstrates that under normoxic or slightly hyperoxic conditions [partial pressure of O_2 (PO_2) = 100–150 mmHg], basal activity of human TRPA1 channels expressed in HEK cells is low. Elevated PO_2 (maximal response at $PO_2 \approx 250$ mmHg) increases TRPA1 activity by a mechanism that requires oxidation of specific cysteine residues (C633 and C856). Takahashi and co-workers also demonstrated that moderate levels of hypoxia (maximal response at $PO_2 \approx 70$ mmHg) activate human TRPA1 channels in an HEK expression system. Hypoxia-induced activation of TRPA1 may result from diminished activity of prolyl hydroxylase (PHD) enzymes. PHD requires O_2 as a co-factor for enzymatic activity, and a reduction in PO_2 results in diminished hydroxylation of proline residues at specific recognition sites. Block of PDH activity stimulates TRPA1 activity, and hypoxia-induced increases in TRPA1 activity are associated with decreased hydroxylation of proline residue 394 (P394). In addition, TRPA1 protein levels at the cell surface are increased after ~10–15 min exposure to hypoxia. These findings suggest that decreased PO_2 inhibits PHD activity, leading to decreased hydroxylation of P394, which promotes translocation of TRPA1 channel protein to the cell surface. However, it is unclear if TRPA1 channels that are newly inserted into the plasma membrane become spontaneously active or if these new channels are recruited to activity by agonists produced endogenously under hypoxic conditions. Additional work is needed to determine if TRPA1 channels mediate vascular responses to hypoxia.

TRPA1 pharmacology: selective inhibitors

Many substances such as camphor (Macpherson *et al.*, 2006), menthol (Macpherson *et al.*, 2006), ruthenium red (Nagata *et al.*, 2005) and the trivalent ion Gd^{3+} (Nagata *et al.*, 2005) block TRPA1 channels, but are not clinically or experimentally useful because they lack specificity. Fortunately, a number of small molecule inhibitors with excellent selectivity for TRPA1 have recently become available (Table 4). HC-030031 is the most widely used TRPA1 blocker (McNamara *et al.*, 2007), and non-specific effects of the compound have not been reported. In addition, one study demonstrates oral bioavailability of HC-030031 (Eid *et al.*, 2008). Chembridge-5861528 is a conger of HC-030031 (Wei *et al.*, 2009; 2010) with similar potency and specificity, and reportedly better solubility in aqueous solutions. AP-18 acts as a partial TRPA1 agonist, leading to desensitization rather than inhibition (Petrus *et al.*, 2007; Defalco *et al.*, 2010). A-967079 is the most potent of the TRPA1 inhibitors currently available but demonstrates species-specific differences for inhibition of human versus rodent TRPA1 channels (McGaraughty *et al.*, 2010). The potency and selectivity of certain tricyclic 3,4-dihydropyrimidine-2-thione derivatives are quite promising (Gijsen *et al.*, 2012), but these compounds are not yet generally available.

TRPA1 in the vasculature

Nerve-evoked vasodilatation

The first evidence demonstrating a role for TRPA1 channels in vascular tone regulation was reported by Bautista *et al.*, who found that TRPA1 channels are present in adventitial nerve fibres in rat mesenteric arteries, and that AITC, raw garlic extracts, purified allicin and DADS caused relaxation of mesenteric artery rings preconstricted with phenylephrine (Bautista *et al.*, 2005). This response was largely blocked when the tissue was pretreated with ruthenium red, capsaicin or with the calcitonin gene-related peptide (CGRP) receptor agonist $CGRP_{8-37}$, but was not altered by the TRPV1 blocker capsazepine. These findings were later confirmed by Pozgai *et al.*, who reported that CA dilates preconstricted mouse mesenteric artery rings (Pozsgai *et al.*, 2010). The later study demonstrated that the CA-induced dilation was only partially attenuated by removal of the endothelium and was greatly diminished in TRPA1 knockout mice (Pozsgai *et al.*, 2010). Further evidence supporting a role for TRPA1 in local vascular control was provided by a study demonstrating that increases in hind paw blood flow in response to injections of 4-ONE were absent in TRPA1 and CGRP knockout mice, but did not differ between TRPV1 knockout mice and controls (Graepel *et al.*, 2011). A study by Kunkler *et al.* investigated the role of TRPA1 channels in meningeal vasodilatation and reported that AITC, CA and acrolein stimulated the release of CGRP from cultured rat trigeminal neurons. This response was

Table 4

Selective TRPA1 inhibitors

Antagonist	IC_{50}	Reference
AP-18	3.1 μM (human) 4.5 μM (mouse)	Petrus *et al.* (2007); Defalco *et al.* (2010)
A-967079	67 nM (human) 289 nM (rat)	McGaraughty *et al.* (2010)
HC-030031	5.3 ± 0.2–6.2 ± 0.2 μM (human)	McNamara *et al.* (2007)
Chembridge-5861528	14.3 ± 0.7–18.7 ± 0.3 μM (human)	Wei *et al.* (2009, 2010)
Tricyclic 3,4-dihydropyrimidine-2-thione derivatives	As low as ~10 nM (rat and human)	Gijsen *et al.* (2012)

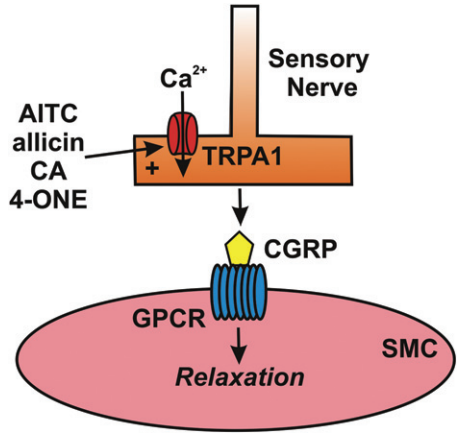

Figure 1

Activation of TRPA1 channels in sensory nerves causes arterial dilation. Allyl isothiocyanate (AITC), allicin, cinnamaldehyde (CA) and 4-oxo-2-nonenal (4-ONE) activate Ca^{2+} influx via TRPA1 channels in sensory nerves, causing release of calcitonin gene-related peptide (CGRP) from perivascular terminals. CGRP binds to its G protein-coupled receptor (GPCR) on the plasma membrane of vascular smooth muscle cells (SMCs) to cause membrane hyperpolarization and myocyte relaxation.

blocked by HC-030031 (Kunkler *et al.*, 2011). Nasal administration of AITC and acrolein stimulated transient increases in meningeal blood flow in rats that was inhibited by $CGRP_{8–37}$ and HC-030031. These studies provide strong evidence supporting the pathway proposed by Bautista *et al.*, who suggested that stimulation of TRPA1 channels present in primary sensory neurons with chemical agonists causes Ca^{2+} influx, leading to localized release of CGRP at perivascular varicosities present on the walls of arteries (Bautista *et al.*, 2005). CGRP binds G protein-coupled receptors on underlying smooth muscle cells to cause membrane hyperpolarization (Hogestatt *et al.*, 2000), myocyte relaxation and vasodilatation (Figure 1). TRPV1 channels, which are commonly co-expressed with TRPA1 in primary sensory neurons (Story *et al.*, 2003), do not appear to be necessary for this response.

TRPA1 channels present in primary sensory neurons may play an important role in the vascular component of neurogenic inflammation. This possibility was first reported by Trevisani *et al.*, who showed that the TRPA1 agonist 4-HNE caused oedema when injected into the hind paws of rats (Trevisani *et al.*, 2007). Additional evidence for an inflammatory role for TRPA1 was provided by a study demonstrating that in guinea pigs, tracheal plasma extravasation resulting from cigarette smoke inhalation was attenuated by inhibition of TRPA1 with HC-030031 (Andre *et al.*, 2008). Furthermore, increases in capillary permeability were absent in TRPA1 knockout mice administered an aqueous extract of cigarette smoke (Andre *et al.*, 2008). *N*-acetyl-*p*-benzoquinone imine (NAPQI), a metabolite produced when large doses of acetaminophen are administered, activates TRPA1 channels and causes tracheal plasma extravasation in rats and mice that is sensitive to HC-030031 and TRPA1 knockout (Nassini *et al.*, 2010). NAPQI application also increased plasma protein extravasation in the skin conjunctiva of rats and mice that was attenuated by TRPA1 blockade or gene knockout (Nassini *et al.*, 2010). These findings suggest that inhibitors of TRPA1 may be useful for treating increased capillary permeability and oedema associated with certain inflammatory conditions.

Endothelium-dependent vasodilatation

My laboratory reported that TRPA1 channels are present in the endothelium of rat cerebral and cerebellar pial arteries. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Earley *et al.*, 2009; McGrath *et al.*, 2010). More interestingly, expression of TRPA1 channel protein in this tissue is most abundant in projections of the endothelial cell plasma membrane that penetrates the internal elastic lamina and terminates proximal to vascular smooth muscle cells. These structures are called myoendothelial junctions (MEJs) and have recently been shown to house signalling complexes mediating endothelium-dependent hyperpolarization and vasodilatation of resistance vessels (Sandow *et al.*, 2006; Ledoux *et al.*, 2008). The detailed structure of MEJ signalling complexes was first reported by Sandow *et al.*, who demonstrated that expressions of intermediate conductance Ca^{2+}-activated K^+ channels ($K_{Ca}3.1$) and connexin proteins 40 and 37 are enriched in the MEJs of rat mesenteric arteries (Sandow *et al.*, 2006). Gap junctions between endothelial and smooth muscle cells (myoendothelial gap junctions) are present in MEJs, consistent with enriched expression of connexin proteins in these structures. A number of earlier studies demonstrate the importance of myoendothelial gap junctions in endothelium-dependent hyperpolarization (EDH) and vasodilation (Yamamoto *et al.*, 1998, 1999; Sandow and Hill, 2000; Griffith *et al.*, 2002; Sandow *et al.*, 2002). Work from Ledoux *et al.* confirmed that $K_{Ca}3.1$ expression is enriched in MEJs of mouse mesenteric arteries and also showed that segments of the endothelial cell endoplasmic reticulum (ER) are present in these structures (Ledoux *et al.*, 2008). Furthermore, this study showed that inositol trisphosphate receptor (IP_3R) expression is enhanced in MEJs. More interestingly, MEJs are the sites of transient, spontaneous, subcellular Ca^{2+} signals in the endothelium (Ca^{2+} pulsars) that are generated by Ca^{2+} released from the ER via IP_3Rs. These Ca^{2+} signals hyperpolarize the endothelial cell plasma membrane by activating proximal $K_{Ca}3.1$ channels present in MEJs. Ledoux *et al.* proposed that membrane hyperpolarization is conducted to underlying smooth muscle cells via myoendothelial gap junctions to elicit EDH of the sarcolemma and, ultimately, endothelium-dependent vasodilatation (Ledoux *et al.*, 2008). My laboratory added to this mechanistic concept when we reported that both TRPA1 and $K_{Ca}3.1$ channels are concentrated in MEJs of rat cerebral resistance arteries (Earley *et al.*, 2009). Our study also shows that AITC elicits smooth muscle cell hyperpolarization and concentration-dependent dilation of cerebral arteries that is blocked by disruption of the endothelium, inhibition of TRPA1 with HC-030031, as well as antagonists of $K_{Ca}3.1$, small conductance Ca^{2+}-activated K^+ channels ($K_{Ca}2.3$) and inwardly-rectifying K^+ (K_{IR}) channels. More interestingly, we found that much higher concentrations of AITC are required to provoke global increases in intracellular Ca^{2+} in native, acutely isolated cerebral artery endothelial cells (EC_{50} = 400 μM) compared with the levels of AITC required to cause endothelium-dependent

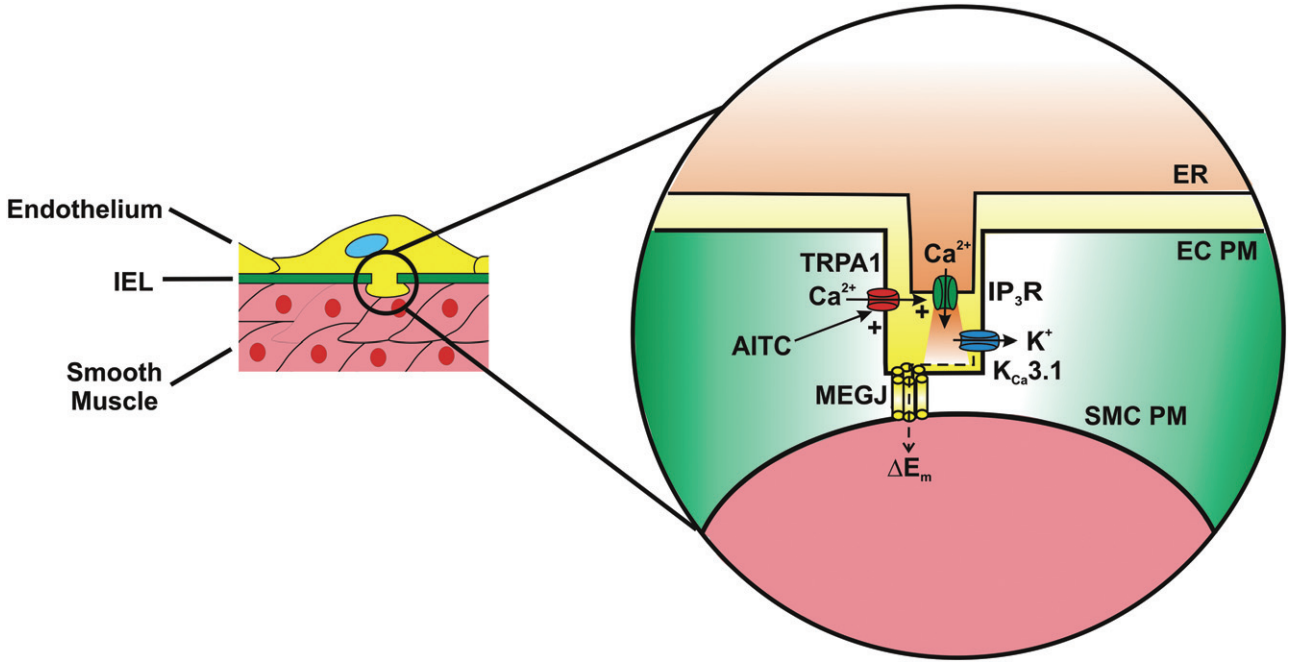

Figure 2

Activation of TRPA1 in cerebral arteries causes endothelium-dependent vasodilation. Allyl isothiocyanate (AITC) activates Ca^{2+} influx via TRPA1 channels present in myoendothelial junctions in cerebral arteries. TRPA1-mediated Ca^{2+} influx stimulates Ca^{2+} release from the endoplasmic reticulum (ER) via inositol trisphosphate receptors (IP_3R). The resulting Ca^{2+} signal (i.e. Ca^{2+} pulsar) stimulates proximal intermediate conductance Ca^{2+}-activated K^+ channels ($K_{Ca}3.1$), resulting in hyperpolarization of the endothelial cell plasma membrane (EC PM). The change in membrane potential (ΔE_m) is conducted via myoendothelial gap junctions (MEGJs) to hyperpolarize the vascular smooth muscle cell plasma membrane (SMC PM), resulting in myocyte relaxation.

vasodilatation (EC_{50} = 16.4 μM) (Earley *et al.*, 2009; 2010). Hence, we proposed a mechanism in which AITC-induced Ca^{2+} influx via TRPA1 stimulates Ca^{2+} pulsar activity within MEJs by Ca^{2+}-induced Ca^{2+}-release, thereby increasing the activity of proximal $K_{Ca}3.1$ channels to hyperpolarize the endothelial cell plasma membrane (Figure 2). This response is presumably transmitted to underlying arterial myocytes via myoendothelial gap junctions to elicit hyperpolarization. K_{IR} channels in smooth muscle cells likely participate in this response by amplifying the initial smooth muscle cell hyperpolarizing stimulus (Smith *et al.*, 2008). The role of $K_{Ca}2.3$ channels is not completely clear, but these channels may play a secondary role to $K_{Ca}3.1$ channels, or may be important for setting the resting membrane potential of the endothelium (Taylor *et al.*, 2003). This proposed mechanism is supported by recent preliminary results demonstrating increased dynamic Ca^{2+} signalling activity in the endothelium of intact cerebral arteries in response to AITC (Taylor *et al.*, 2012).

Endothelial cell expression of TRPA1 has not been reported outside of the cerebral circulation. Preliminary experiments from my laboratory failed to detect TRPA1 expression in the endothelium of rat mesenteric and renal interlobar arteries (unpublished findings) and investigators at the University of Vermont did not record AITC-induced cation currents in patch clamp experiments using native mouse endothelial cells from mesenteric arteries (M.T. Nelson, pers. comm.). Thus, it appears that TRPA1 channels may only be present and functional in the endothelium of certain vascular beds. The significance of this expression pattern is unclear, but is consistent with the possibility of distinct regulatory roles for TRPA1 channels in different segments of the vasculature.

Smooth muscle cells

There is little evidence indicating that TRPA1 agonists elicit vasodilatation by acting directly on TRPA1 channels in vascular smooth muscle cells. Indirect support for such a response was presented by Yanaga *et al.* examining the vasorelaxant effects of CA on precontracted rat aortic rings (Yanaga *et al.*, 2006). CA-induced dilation was impaired but not abolished by removal of the endothelium or by inhibition of NOS. The endothelium-independent component of the response is suggestive that CA can cause vasodilatation of conduit arteries through direct effects on smooth muscle cells. However, this study did not examine the effects of TRPA1 inhibition on CA-induced dilation of aortic ring segments and did not demonstrate expression of TRPA1 in this tissue. Studies from my laboratory did not detect smooth muscle expression of TRPA1 in immunolabelled intact rat cerebral resistance arteries or enzymatically isolated cerebral arterial myocytes (unpubl. obs.), nor did we detect Ca^{2+} influx in native rat cerebral artery smooth muscle cells in response to AITC (Earley *et al.*, 2010). The differences in our findings versus those of Yanaga *et al.* may reflect differential expression of TRPA1 channels in the aorta versus cerebral resistance

arteries, or may be due to TRPA1-independent effects of CA on rat aortic rings.

The big picture: influence of TRPA1 on integrative cardiovascular physiology

It is clear that TRPA1 channel activity can elicit dilation of peripheral arteries through nerve-mediated release of CGRP (Figure 1) or through endothelium-dependent smooth muscle cell hyperpolarization in cerebral arteries (Figure 2). But what effect do these responses have on overall cardiovascular function? Examination of global TRPA1 knockout mice indicates that resting blood mean arterial pressure (MAP) and heart rate (HR) of anaesthetized TRPA1 knockouts do not differ from wild-type controls (Pozsgai *et al.*, 2010), suggesting that mice are able to compensate for lack of TRPA1 activity and maintain basic cardiovascular function in the absence of physiological stressors. However, the effects of TRPA1 knockout on the regulation of MAP and cardiac output in conscious mice at rest or under conditions requiring dynamic cardiovascular regulation have not been reported.

The effects of systemic TRPA1 activation were investigated by Pozagai *et al.*, who reported that in anaesthetized female CD-1 mice, i.v. administration of CA has bimodal effects, causing a short-term fall in MAP and HR (depressor response), followed by a sustained rise in both parameters (pressor response). The depressor response was independent of the CA concentration administered, but the pressor response was concentration dependent (EC_{50} = 78.6 μM·kg^{-1} body weight). Further experiments were performed using male and female knockout mice of mixed genetic background. The drop in MAP and HR in response to a moderate dose of CA (80 μM·kg^{-1} body weight) was diminished in TRPA1 knockout animals compared with controls; however, the pressor response was not observed in the control strain used for these experiments at this concentration. A higher concentration of CA (320 μM·kg^{-1} body weight) elicited both pressor and depressor responses in wild-type mice, and the pressor, but not depressor, response was blunted in the TRPA1 knockout animals. Using TRPV1 and CGRP knockout mice as well as an inhibitor of substance P, the authors demonstrate lack of involvement of these pathways in both the pressor and the depressor responses, suggesting that sensory nerve-derived vasodilators are not involved in systemic responses to i.v. CA administration. Cholinergic receptor blockade (atropine) reduced CA-induced HR decrease, diminished the depressor response to the lower concentration of CA, but had little effect on the pressor response. Block of α-adrenergic receptors attenuated both pressor and depressor responses, but ganglionic blockade (hexamethonium) combined with atropine did not influence the pressor response. The authors conclude that the depressor response results from vasovagal reflex activation, whereas the pressor response is mediated by peripheral sympathetic activation and release of noradrenalin. It is difficult to draw firm conclusions regarding the depressor effects of i.v. CA administration from this report, as the response was very transient in nature (~1 min), there was no relationship between the dose of CA administered and the drop in MAP and HR, and, when the higher concentration of CA was administered, the depressor response did not differ between wild-type and TRPA1 knockout mice. Interpretation of CA-induced pressor responses is confounded by the reported differences in sensitivity to CA among the strains of mice that were used. Female CD-1 mice exhibited concentration-dependent increases in blood pressure in response to CA (EC_{50} ≈ 80 μM·kg^{-1} body weight), but this concentration did not induce pressor responses in the mixed background TRPA1 knockout mice and littermate controls used for the study. Consequently, a CA concentration (320 μM·kg^{-1} body weight) that produced maximal pressor responses in CD-1 mice was used for the experiments employing knockout animals. To better understand the significance of the channel in regulation of cardiovascular homeostasis, further experiments examining the systemic effects of TRPA1 agonist administration are warranted. The consequences of longer-term administration of TRPA1 activators and inhibitors on cardiovascular responses of conscious animals will be of particular interest.

A study by Koda *et al.* measured increases in renal sympathetic nerve activity (RSNA) in response to intra-arterial injection of AITC in decerebrate rats (Koba *et al.*, 2011). This response was prevented by sectioning the sciatic nerve, indicating a muscle-based autonomic reflex response. Maintained (30 s) static contraction of hindlimb muscle induced by electrical stimulation resulted in elevated blood pressure and RSNA activity that was diminished by HC-030031. Injections of metabolic by-products of muscular contraction (arachidonic acid, bradykinin and diprotonated phosphate) resulted in increased RSNA that was diminished by HC-030031. The authors conclude that activation of TRPA1 channels present on muscle afferents by the by-products of muscle contraction contributes to increased sympathetic nervous activity during exercise. This response may contribute to exercise-induced increases in HR and respiration, but additional work is needed to test this hypothesis.

Concluding remarks

Investigation of TRPA1 channels in the vasculature is in an early stage and its definitive role and significance in cardiovascular regulation remains uncertain. It is clear that TRPA1 channels are present in the vascular wall and activation with chemical agonists causes vasodilatation. However, further investigation is needed to determine what physiological and/or pathophysiological situations lead to activation of TRPA1 channels in the vasculature. Activation of TRPA1 channels by substances found in garlic and mustard oil has prompted speculation that this response mediates putative cardioprotective benefits of these foods (Bautista *et al.*, 2005; Earley *et al.*, 2009), but this has not been demonstrated experimentally. In any case, it is unlikely that sensation of dietary molecules is the primary function of TRPA1 channels in the vasculature. It seems much more likely that oxidative stress and hypoxia are the endogenous regulators of TRPA1 channels in resistance arteries. For example, TRPA1 channels in the endothelium of the cerebral circulation may directly sense and mediate arterial dilation in response to changes in PaO_2 associated with impaired oxygenation, but this has not been demonstrated. It is also conceivable that substances such as 4-HNE and 4-ONE that are produced during oxidative stress associated with reperfusion following ischaemia and other conditions cause arterial dilation through activation of TRPA1 channels in the endothelium. However, this putative

response requires generation of highly reactive superoxide anions (O_2^-) responsible for lipid peroxidation proximal to TRPA1 channels in the vascular wall by an unknown mechanism. Thus, considerable additional investigation is necessary to determine endogenous signalling pathways and physiological roles of TRPA1 in healthy and disease arteries. In addition, the significance of the channel's differential pattern of expression (perivascular nerves vs. MEJs) in various vascular beds also awaits discovery. Such studies will likely require tissue-specific and/or inducible TRPA1 knockout models. These are critically important issues, as TRPA1 channels are under investigation for pharmaceutical development, particularly for the treatment of chronic pain, and the long-term effects of TRPA1-modifying agents on the cardiovascular system in humans are unknown. Most importantly, it is not known if TRPA1 channels are potential targets for the development of new therapies for the treatment or prevention of cardiovascular disease.

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Conflict of interest

No conflicts.

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