Cinnamaldehyde: evidence of TRPA1-dependent vasodilator responses in the peripheral vasculature *in vivo* via a CGRP and nitric oxide-sensitive mechanism

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Transient receptor potential ankyrin 1 (TRPA1) are widely expressed on primary sensory neuron endings and have a polymodal activation, including cold temperature, pungent products from vegetable, reactive oxygen species and mechanical stimuli (Aubdool & Brain, 2011; Ryckmans et al., 2011). Our group has previously shown that TRPA1 is involved in mediating peripheral vasodilatation (Graepel et al.,2011) and can also influence changes in blood pressure of possible relevance to autonomic system reflexes (Pozsgai et al., 2010). Here, we used pharmacological inhibitors and genetically modified mice to investigate the possible mechanisms involved in TRPA1 agonist-mediated vasodilatation using the mouse ear model.

Cutaneous blood flow was measured in mice (20-30g) anaesthetised with ketamine (75mg/kg) and medetomidine (25mg/kg) *i.p.* using non-invasive laser Doppler flowmeter where blood flow is measured in a focussed restricted area (1-2mm^2) of the ear or Full-Field laser perfusion imager where average blood flow is assessed in the whole ear. 20µl of cinnamaldehyde (10%) and vehicle (10% DMSO in ethanol) was applied topically on the ipsilateral and contralateral ears, respectively and blood flow was recorded for 30 min. All animals were randomly assigned to drug-treated or their respective control groups. Results were expressed as mean $\pm$ s.e.m. in arbitrary flux units (x10^3 flux units), and analysed by 2-way ANOVA + Bonferroni's test.

Initial studies showed that cinnamaldehyde significantly increased ear blood flow, as compared to vehicle-treated ears (176.2 $\pm$ 27.9 vs 35.0 $\pm$ 0.7 (x10^3), $p<0.01$, n=5) and this response was significantly attenuated in TRPA1 KO mice on a mixed background (n=3-4, $p<0.01$). A similar trend was observed in mice pre-treated *i.p. 30 min* with the TRPA1 antagonists HC030031 (100mg/kg, n=5) or TCS5861528 (10mg/kg, n=5). The selectivity of cinnamaldehyde for another TRP channel, TRPV1 was also tested and TRPV1 deletion did not affect cinnamaldehyde-induced vasodilatation, as shown in TRPV1 KO mice, on a C57BL/6 background (n=5). We conclude that cinnamaldehyde causes vasodilatation by selectively activating TRPA1 and not TRPV1 *in vivo.*

Furthermore, our data showed that cinnamaldehyde-induced vasodilatation involves a neurogenic component where CGRP KO mice on a C57BL/6 background (n=3) and WT mice pre-treated with CGRP receptor antagonist $CGRP_{8-37}$ (400nmol/kg, *i.v.*, n=5) displayed significantly lower vasodilatation than their respective control pre-treated groups ($p<0.01$, $p<0.0001$ respectively). We also observed a significant decrease in responses with WT pre-treated with a combination of $CGRP_{8-37}$ and neurokinin-1 receptor antagonist SR140333 (480nmol/kg, *i.v*, n=5, $p<0.001$)*).* Although the cyclo-oxygenase inhibitor indomethacin (5mg/kg, *s.c. 30 min*, n=3) had no effects on cinnamaldehyde-induced vasodilatation, we observed that neuronal nitric oxide synthase (nNOS) inhibition by SMTC (10mg/kg, *i.v.* n=6) caused a significant decrease in cinnamaldehyde-induced blood flow responses ($p<0.001$). A cocktail treatment of $CGRP_{8-37}$, SR140333 and SMTC (*i.v. n=5-6)* significantly attenuated cinnamaldehyde-induced vasodilatation in WT mice ($p<0.0001$).

We provide novel evidence that topical cinnamaldehyde administration activates TRPA1 and thus promotes cutaneous vasodilatation which is dependent on the release of the potent vasodilator neuropeptide CGRP but not on prostaglandins. This study also highlights that cinnamaldehyde-induced vasodilatation may also involve increased neuronal nitric oxide release. These results suggest that CGRP and nNOS isoforms are both particular important in TRPA1-dependent vascular effects *in vivo*.

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This study was supported by a BBSRC-led IMB capacity building award.