190

THE PARTICIPATION OF REACTIVE OXYGEN SPECIES AND TRPA1 IN CINNAMALDEHYDE-INDUCED VASODILATATION IN THE PERIPHERAL VASCULATURE

A Aubdool, X Kodji, E Fernandes, S Bevan, S D Brain *King's College London*

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The non-selective transient receptor potential ankryrin-1 (TRPA1) channels have been previously reported to be a major chemosensory receptor for reactive oxidants in sensory neurons in both in vivo and in vitro studies, but its importance to the vascular field has yet to be investigated. We have recently shown that cinnamaldehyde can activate TRPA1 and release potent microvascular vasodilators neuropeptide CGRP and nitric oxide (Aubdool et al., 2012a, 2012b) and, causes vasodilatation. We hypothesised that reactive oxygen species (ROS) are involved in the downstream signalling mechanism and this was investigated using the mouse ear model. Using laser Doppler flowmetry, cutaneous blood flow was

measured in male CD1 mice (20–25g) under anaesthesia (ketamine-75mg/kg; medetomidine-25mg/kg, i.p.) and following topical application of cinnamaldehyde (10%) and vehicle (10% DMSO in ethanol). Ear samples were collected and hydrogen peroxide (H2O2) levels were assessed using the amplex red assay. All animals were randomly assigned to drug-treated or respective control groups. Results were expressed as mean+S.E.M. in arbitrary flux units and analysed by 2-way ANOVA+Bonferroni's test. A sustained (30 min) increase in vasodilatation was observed after topical application of cinnamaldehyde and this was significantly attenuated by a treatment of ROS scavenger N-acetylcysteine (NAC, 300mg/kg), (379.0+40.1x103 flux units for control-treated vs 168.4+24.2x103 flux units for NAC-treated, n=5-6, $p<0.001$). No change in H2O2 levels was observed in cinnamaldehyde-treated ears compared to vehicle. There was no significant change in cinnamaldehyde-induced vasodilatation in vehicle or NADPH oxidase inhibitor apocynin (20mg/kg, i.v.) pre-treated wild-type (WT) mice (n=6, $p>0.05$). However, TRPA1-mediated vasodilatation was significantly reduced by a co-treatment of superoxide dismutase (SOD) and catalase (25000U/kg, $p<0.01$, n=6), but not deactivated enzymes, supporting a novel role for ROS generation. This response was also attenuated in WT mice pre-treated with the iron chelator deferoxamine (25mg/kg, $p<0.001$, n=6) or the permeable SOD mimic TEMPOL (30mg/kg, $p<0.001$, n=6), suggesting a potential involvement of hydroxyl radicals and oxidative stress. These studies provide novel evidence that ROS are involved in TRPA1-dependent neurogenic vasodilatation in vivo. Aubdool AA et al (2012a). BPS Focused Meeting on Neuropeptides 028P pA2 Online. Aubdool AA et al (2012b). BPS Winter Meeting Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst156P.pdf This study was supported by a BBSRC-led IMB capacity building award.