Investigating the effects of noxious skin heating on cutaneous blood flow in TRPV1 Wild Type & TRPV1 $^{-/-}$ mice.

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The transient potential receptor vanilloid 1 (TRPV1) receptor is a non-selective cation channel that is activated by capsaicin, (the pungent component of hot peppers), temperatures within the noxious range (>43 degrees C) and low pH (<pH 6.0). TRPV1 receptors are expressed in primary afferent fibres, such as A-delta and C-fibres, and non-neuronal cells such as vascular smooth muscle. Capsaicin-induced TRPV1 activation on sensory nerves leads to vasodilation via neuropeptide release, whereas TRPV1 activation on vascular smooth muscle causes vasoconstriction (Kark et al., 2008). In the present study, we investigated the effects of local heating on cutaneous blood flow in the ears of TRPV1 wild type and knockout mice utilising a novel laser Doppler monitor integrated with a skin heating unit. The effect of the TRPV1 antagonist, AMG9810, was also investigated using this model in CD1 mice. AMG9810 has previously been shown to attenuate capsaicin-induced vasodilation of ear pinnae, cause hyperthermia, decrease baseline skin blood flow in mice and increase thermal withdrawal latencies of mice (Alawi et al., 2012).

Male & female, age matched TRPV1 Knockout (KO) and Wild Type (WT) mice (25-30 g of body weight) and CD1 strain mice (30-35 g of body weight, Charles River UK) were used in all experiments, in accordance with the UK Scientific Procedures Act 1986. Acute changes in peripheral blood flow in response to local heating in the pinnae of the ears of anaesthetised mice (ketamine 75mg/kg & medetomidine 25mg/kg; *i.p.*) was investigated utilising the moorVMS-HEAT

Skin Heater Module (VHP2) with an integrated P3 needle probe connected to the moorVMS-LDF Laser Doppler blood flow and temperature monitor (Moor Instruments, UK). Baseline blood flow was obtained for 5 min and then the ears were heated to 45°C for a duration of 5 min, in 0.1°C/sec increments; blood flow was monitored following the cessation of heating for 30 min. In separate studies, CD1 mice were pre-treated with AMG9810 (50mg/kg) or vehicle and the effects of local skin heating was investigated.

Heating of the ears resulted in a profound increase in cutaneous blood flow, which was significantly attenuated in TRPV1 KO mice ($p>0.001$ between 4-10 min post-heating) vs. TRPV1 WT mice. Interestingly, AMG9810 did not result in any attenuation of the significant increase in blood flow in the ears of CD1 mice in comparison to vehicle-treated mice ($p<0.05$). All statistics were determined by 2-way ANOVA.

In conclusion, we have demonstrated that noxious heat increases skin blood flow in mice in a TRPV1-related manner. Further studies are required to determine why the effect on blood flow was attenuated in TRPV1 knockout mice, but not in AMG9810-treated mice. It may be that the effect of heating on blood flow is not directly TRPV1 dependent and the attenuated effect in knockout mice is due to compensatory effects in the knockout mice.

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