A characterisation of the hyperthermic, anti-nociceptive and vascular effects of the TRPV1 antagonist, AMG9810.

Khadija Alawi, Amelia Dyer, Julie Keeble. *King's College London. Institute of Pharmaceutical Science, School of Biomedical Sciences, London, UK*

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 (Kark et al., 2008).TRPV1 antagonists are analgesic, but have recently been shown to cause an increase the body temperature, as seen in man, rodents and monkeys (Gavva et al., 2007). The precise mechanism underlying the hyperthermia is, however, unknown. This study aimed to characterise the hyperthermic, anti-nociceptive and vascular effects of the TRPV1 antagonist, AMG9810.

Male and age matched TRPV1 Knockout (KO) and Wild Type (WT) mice were used in all radio-telemetry studies (30-40 g of body weight); male CD1 mice (30-35 g of body weight, Charles River UK) were used in all other experiments, in accordance with the UK Scientific Procedures Act 1986. AMG9810 (Gavva et al., 2005), a selective TRPV1 antagonist (50mg/kg; *i.p.*) or its vehicle control (2% DMSO, 5% Tween 80 in sterile saline; 10ml/kg), was administered to TRPV1 KO and WT mice implanted with radio-telemetry transmitters to monitor core body temperature and activity for 24 h following a 1 h baseline recording (ambient temperature 22 ± 2 ºC). Radio-telemetry surgical implantations were undertaken one week prior to drug administration. Buprenorphine (10µg/kg, *i.m.*) was administered 10 min prior to surgery. The mice were anaesthetised and the radio-telemetry transmitter (TA10TA-F20; DSI, St Paul, MN, USA) was inserted into the abdominal cavity. Surgery was performed under isoflurane anaesthesia (2-3% vol. isoflurane; 2-3% vol. O_2). In separate studies CD1 mice were pre-treated with AMG9810 (50mg/kg) or vehicle and thermal withdrawal latencies were investigated using a modified Hargreaves' test for mice. Acute changes in peripheral blood flow in anaesthetised mice (ketamine 75mg/kg & medetomidine 25mg/kg; *i.p.*) were investigated utilising a Laser Doppler Perfusion imagery (moorFLPI, Moor Instruments, UK) following AMG9810 (50mg/kg; *i.v.*) or vehicle treatment; mice were monitored for 1 h post injection. Additionally, capsaicin-induced blood flow changes were investigated in the pinnae of the ears (20 µl, 10mg/ml dissolved in 100% ethanol) and the exposed synovial membrane of the knee joints (10 µl, 100nmmol, dissolved in 100% ethanol). In these experiments, capsaicin was applied to the ipsilateral ear/knee joint whilst ethanol (100%) was used as a control for the contralateral ear/knee joint.

AMG9810 (50mg/kg) induced a significant increase in the body temperature of WT mice at 1 h post-treatment but not in TRPV1 KO mice (+ 1.13 °C ± 0.14 °C WT vs. + 0.22 °C ± 0.3 °C KO, $p<0.001$, n= 3-4). Additionally, AMG9810 increased the thermal withdrawal latencies of mice at 2 h post-treatment ($p<0.01$, n=6). Furthermore, AMG9810 was able to significantly attenuate the vasodilation induced by capsaicin application to the ears of mice ($p<0.001$) whilst the vasoconstriction observed in the synovial membrane of the knee joint was not significantly different between AMG9810 and vehicle treated animals ($p>0.05$). AMG9810 resulted in a decrease in blood flow in several regions such as the legs and paws that was

significant from 8-10 mins post-injection ($p<0.001$; legs, $p<0.01$; paws, $n=6$). All statistics are determined by 2-way ANOVA.

In conclusion, AMG9810 induced a significant hyperthermia which is selective to TRPV1, demonstrated by lack of effect in TRPV1 KO mice. In addition, we demonstrated that AMG9810 was able to increase the withdrawal latencies of mice to noxious heat, in comparison to control treated mice. Additionally, AMG9810 attenuated capsaicin-induced vasodilation in the pinnae of the ears, whilst the vasoconstriction observed in the exposed synovial membrane was not attenuated by AMG9810 treatment. The latter effect could be due to non-neuronal TRPV1 receptor activation, of which the precise mechanisms remain to be elucidated. Finally, we observed an acute decrease in blood flow in several regions in anaesthetised mice in response to AMG9810. This decreased cutaenous blood flow is a possible mechanism contributing to the hyperthermia induced by AMG9810.

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