Investigating the expression of uncoupling proteins in TRPV1 antagonist-mediated hyperthermia

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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. TRPV1 antagonists cause hyperthermia, as seen in man, rodents and monkeys (Gavva et al., 2007). The hyperthermia is accompanied by thermogenesis (for review see Romanovsky et al., 2009). Furthermore, repeated administration of TRPV1 antagonists has been shown to attenuate hyperthermia. This study therefore (1) examined thermogenic protein expression (mitochondrial uncoupling protein 1(UCP1) in mouse brown adipose tissue (BAT) as this tissue is the main site of thermogenesis in rodents (2) the effect of repeated administration of the TRPV1 antagonist, AMG9810 (Gavva et al., 2005). Therein, we aimed to determine whether regulation of UCP expression underlies attenuation of TRPV1-antagonist-induced hyperthermia.

Mice (CD1 strain, 30-35 g of body weight & C57BL/6, 25-30 g of body weight, Charles River UK) were used in all experiments, in accordance with the UK Scientific Procedures Act 1986. Mice undergoing repeated administration of AMG9810 (n = 4, 50mg/kg; *i.p.)* or vehicle (n=4, 2% DMSO, 5% Tween 80 in sterile saline; 10ml/kg) were 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 CD1 mice, AMG9810, (n= 6, 50mg/kg; *i.p.*) or its vehicle control (n= 6, 2% DMSO, 5% Tween 80 in sterile saline; 10ml/kg) was administered and animals were culled 4 h post-treatment andintrascapular BAT was collected for messenger RNA and protein analysis. Total RNA from iBAT samples was extracted using the *RNeasy® Microarray Tissue Mini kit* containing *QIAzol* Lysis Reagent (Qiagen), following the manufacturer's instructions. Total RNA [1µg] was reverse transcribed in a 20-µl reaction volume with the *High Capacity RNA-to-cDNA Kit* (Applied Biosystems ®) according to the manufacturer's instructions. Target genes were amplified using a real-time PCR cycler, Rotor-Gene 6000 (Qiagen). For immunoblotting analysis, the samples were processed utilising the *Qproteome mitochondria isolation kit* (Qiagen UK). Tissue was separated into cytosolic, nuclei, microsomal and mitochondrial protein fractions and protein expression of UCP1 was investigated.

AMG9810 caused significant hyperthermia that was not attenuated by repeated administration ($p < 0.01$ AMG9810 (Day 1) vs. Vehicle at 2 h post-treatment (37.08 ± 0.23 °C vs. 35.77 ± 0.04 °C, respectively)). Repeated treatment of AMG9810 did not attenuate the hyperthermia throughout days 1 – 4 in the study ($p < 0.05$). Brown adipose tissue samples from AMG9810-treated mice at 4 h post-treatment demonstrated a trend towards a reduction of UCP1 protein in comparison to vehicle-treated mice, both in the mitochondrial fraction and overall in all combined fractions. Interestingly, the results obtained from RT-PCR experiments indicated a significant a reduction in the expression of UCP1in AMG9810 treated mice ($p > 0.05$), compared to vehicle treated mice.

In conclusion, there is an intriguing lack of attenuation of AMG9810-induced hyperthermia with repeated administration. Furthermore, additional studies are required to determine whether uncoupling proteins do play a role in TRPV1 antagonist-induced hyperthermia. This will involve the use of alternative TRPV1 antagonists and knockout mice.

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