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Neuroscience Letters 401 (2006) 55–58
Neuroscience Letters
www.elsevier.com/locate/neulet

Capsaicin-induced vasoconstriction in the mouse knee joint: A study using TRPV1 knockout mice

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Received 13 October 2005; received in revised form 7 February 2006; accepted 22 February 2006

Abstract

Capsaicin is the pungent component of chilli peppers that concomitantly activates and desensitizes C-fibre and Aδ sensory nerve fibres. Stimulation causes an acute neurogenic response including vasodilation, plasma extravasation and hypersensitivity. However, in the present study we have shown that capsaicin produces a dose-dependent vasoconstrictor effect in the mouse knee joint via Transient Receptor Potential Vanilloid 1 (TRPV1) receptor activation. A ^{125}I-albumin accumulation technique showed that the intravascular volume of capsaicin-treated joints in wild type (WT) mice was significantly reduced compared to TRPV1 knockout mice ($p < 0.01$). Similarly, a laser Doppler technique showed significantly reduced blood flow in the capsaicin-treated joints of WT compared to TRPV1 knockout mice ($p < 0.001$). Pretreatment with guanethinidine (50 mg kg^{-1}, i.p.) had no effect on the vasoconstriction. These data are important considering the involvement of TRPV1 receptors in joint disease. The mechanisms underlying the vasoconstriction therefore require further investigation.

Keywords: Capsaicin; Sensory nerves; Neuropeptides; Blood flow; Intravascular volume

Capsaicin (*N*-vanillyl-nonenamide) is a small, pungent molecule derived from hot chilli peppers that is known to concomitantly stimulate and desensitize a subpopulation of C-fibre polymodal nociceptive and Aδ sensory nerve fibres via activation of the transient receptor potential vanilloid 1 (TRPV1) receptor [7]. TRPV1 is a member of the family of TRP cation channels and other agonists of this receptor include noxious heat (>43 °C), protons and endogenous lipids such as anandamide and lipoxygenase products [23]. TRPV1 receptor activation causes release of neuropeptides (substance P and calcitonin gene-related peptide, amongst others) from sensory nerves, eliciting a neurogenic inflammation in the surrounding area. Predominant neurogenic responses include vasodilation, plasma extravasation and hypersensitivity. Prolonged exposure to capsaicin depletes the neuropeptide content of the nerve terminals, producing the desensitization response [12,13].

Previous studies in our laboratory have demonstrated that capsaicin produces a vasodilator response in the mouse ear, as expected [9]. The capsaicin-induced vasodilation in the ear is due to a combination of substance P and calcitonin gene-related peptide (CGRP) [9,10], CGRP being one of the most potent vasodilating substances known [3]. More generally, CGRP, acting via CGRP1 receptors, is considered to be the principal transmitter of neurogenic vasodilation [2] whereas substance P, acting via neurokinin 1 (NK1) receptors, mediates increased vascular permeability [17].

In the present study, vascular responses to capsaicin in the mouse knee joint have been investigated. Previous studies have suggested that capsaicin can elicit a vasoconstriction in the rat knee joint via an unknown mechanism [4]. Here we have examined the vasoactive response to capsaicin in WT and TRPV1 knockout mice. Experiments have been performed in mice, using ^{125}I-albumin to measure intravascular volume changes following intra-articular injection of capsaicin. The direct measure of blood flow was achieved through use of a laser Doppler technique.

Female CD1 mice (25–30 g) were purchased from Harlan (UK). Female C57BL6/129SVJ mice (25–30 g), either genetically unaltered wild type (WT) or knockout mice lacking the gene for the TRPV1 receptor (TRPV1KO), were bred in house from mice donated by S Boyce (Merck, Sharp & Dohme, UK). Original breeding pairs were generated by D Julius (University of California, San Fransisco, UK), as described previously [6]. The phenotype of these mice has been well characterized

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doi:10.1016/j.neulet.2006.02.083

previously [15]. All mice were kept in a climatically controlled environment and had access to food and water ad libium. All procedures were in accordance with the UK Home Office Scientific Procedures Act (1986). Mice were anaesthetised with urethane (2.5 mg g^{-1}, i.p.) and 30 G needles were used for all intra-articular injections (BD Micro-Fine insulin syringes, 0.3 ml). Chemicals were obtained from Sigma Aldrich (Dorset, UK), unless otherwise stated.

CD1 mice were used to determine the dose-dependency of vascular responses to capsaicin in the joint using a ^{125}I-albumin accumulation technique. ^{125}I-albumin (45 kBq, ICN, UK) was administered intravenously via the tail vein and allowed to re-circulate. Mice then received intra-articular injections of capsaicin (0.1–100 nmol; 10 μl) and vehicle (80% saline, 10% ethanol, 10% Tween 80; 10 μl; contralateral joint) into the knee joint. A control group received vehicle into both joints. At 2, 10, 20 and 30 min thereafter, ^{125}I-albumin levels in the joint were determined using a collimated gamma probe (Europrobe, Bright Technologies, UK; for details see [14]). In short, the head of the probe was held against the intact knee joint, adjacent to the underlying patellar ligament, and counts/min detected in the joint were measured. The ^{125}I-albumin accumulation was expressed as a percentage of counts/min detected in the agent-treated joint compared to the vehicle-treated joint to allow for unavoidable differences in the quantity of radioactivity injected. One hundred percent indicates that the same quanitity of ^{125}I-albumin is present in both joints and, thus that no vascular events have taken place. A percentage of >100 shows that more ^{125}I-albumin is present in the test joint compared to the control joint. Vice versa, a percentage of <100 shows that less ^{125}I-albumin is present in the test joint compared to the control joint. These experiments were repeated in WT and TRPV1KO mice using a single dose of capsaicin (100 nmol, vehicle injected into contralateral joint).

In separate experiments, CD1 mice were pretreated with either guanethidine (50 mg kg^{-1}) or its vehicle (saline, 10 ml kg^{-1}) 24 h prior to ^{125}I-albumin accumulation experiments to eliminate the possibility that reflex sympathetic responses were affecting the responses to capsaicin. This regime of sympatholytic treatment has been used previously in mice by Malmberg and Basbaum [19] to produce a significant effect in a model of neuropathic pain.

To confirm whether the changes in ^{125}I-albumin accumulation were due to changes in vascular tone as opposed to vascular permeability, further experiments were performed in WT and TRPV1KO mice using a Laser Doppler blood flow Imager (IDI; Moor Instruments, UK). The Doppler probe emits a low intensity laser beam that scans across the tissue surface being assessed, mapping blood flow with a total of 65,536 individual measurements. The probe measures a flux value that is proportional to the number of red blood cells passing through the laser beam multiplied by their velocity. Increased flux is indicative of vasodilation. For these experiments, the skin overlying the knee joints was removed and the patellar tendons dissected to expose the synovial membrane. Pretreatment values were obtained for both joints. To avoid movement of the joint thereafter, capsaicin (100 nmol, 10 μl) and vehicle (100% ethanol, 10 μl; contralateral joint) were applied topically to the synovial membrane. Flux measurements were obtained every minute thereafter for 15 min.

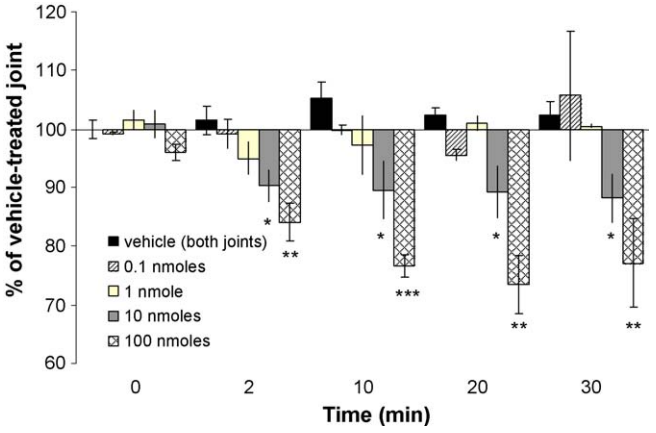

Fig. 1. Changes in ^{125}I-albumin accumulation following intra-articular injection of capsaicin into the CD1 mouse knee joint. The dose response relationship to capsaicin and changes over a 30 min period is shown. $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ (ANOVA two-way analysis of variance, Bonferonni post hoc test; $n=4$–5) compared to vehicle (both joints).

Knee diameter was measured using calipers (Mitutoyo) in one group of CD1 mice to determine whether capsaicin (10–100 nmol) caused swelling of the joint. Measurements were taken before and after (30 min) injection of vehicle and capsaicin.

Intra-articular injection of capsaicin caused a dose-dependent decrease in ^{125}I-albumin in the mouse knee joint compared to the contralateral, vehicle-treated joint (Fig. 1). The decrease in ^{125}I-albumin was rapid (>2 min), indicative of a change in vascular tone rather than vascular permeability [14]. The ^{125}I-albumin technique can detect changes in vascular tone as previously shown in response to endothelin in the mouse knee joint [14]. The reduced ^{125}I-albumin levels in the capsaicin-treated joint did not change relative to the contralateral joint over time, thus showing a sustained decrease in blood flow. The highest dose of capsaicin used (100 nmol) produced a similar response in C57BL6/129SVJ WT mice to that observed in the CD1 mice (Fig. 2). In contrast, the response was completely abolished in

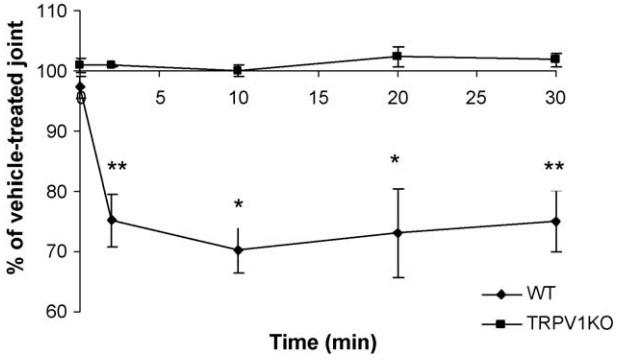

Fig. 2. Changes in ^{125}I-albumin accumulation following intra-articular injection of capsaicin (100 nmol) in wild type (WT) and TRPV1 knockout (TRPV1KO) mice. $^{*}p<0.05$; $^{**}p<0.01$ (ANOVA two-way analysis of variance, Bonferonni post hoc test; $n=3$–5) compared to TRPV1KO.