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Cooling inhibits capsaicin-induced currents in cultured rat dorsal root ganglion neurones

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Abstract

Whole-cell and single-channel recordings from rat dorsal root ganglion neurones were used to investigate the temperature dependence of currents through the capsaicin receptor (vanilloid receptor 1, VR1). Reducing the temperature from 31 to 14°C inhibited the current induced by 0.5 μM capsaicin by 80%. The Q_{10} (temperature coefficient over a 10°C range) of the whole-cell capsaicin-induced current was 2.3 between 10 and 30°C. Single-channel recordings showed that this inhibition by cooling was due to a marked reduction in the open probability ($Q_{10} = 8.2$ between 10 and 30°C). This effect can explain the pain relief and reduction in inflammation caused by strong cooling of the skin.

Keywords: Vanilloid receptor 1 (VR1); Cold; Inflammation; Nociceptor; Pain; Primary sensory neurone

A population of nociceptive primary afferent neurones expresses non-selective cation channels activated by capsaicin (the pungent ingredient of hot chilli peppers), heat in the noxious range (above about 42°C) and acidic solutions. The underlying molecule, VR1 (vanilloid receptor 1), has recently been cloned and expressed [2,13]. Studies in null mutant mice indicate that VR1 has an important role in mediating thermal hyperalgesia during inflammation [1,4] and it is known to stimulate release of the neuroactive peptides CGRP and substance P [5,12]. The stimuli that activate VR1 act synergistically – capsaicin and acids lower the heat threshold for activation [13], while capsaicin-activated currents are augmented by heat, with a Q_{10} of about 2.1 over the temperature range 23–52°C [14]. Inflammatory mediators can also shift the heat threshold for VR1 activation below normal body temperature [9,13]. The observed augmentation of capsaicin-induced currents by heating raises the question of whether the current could also be inhibited by cooling; such an effect could underlie the relief of pain and inflammation observed on application of strong cooling [9]. We carried out the present study to test this hypothesis.

Adult male Wistar rats (200–275 g) were killed by CO_2 inhalation followed by decapitation. Dorsal root ganglia (DRGs) were dissociated with collagenase (0.6–1 mg/ml) and Dispase (3 mg/ml), and cells were plated on poly-D-lysine coated coverslips, and cultured for 1–3 days (37°C, 5% CO_2 in air) in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium supplemented with 10% horse serum and 50 μg/ml gentamicin (all items from Sigma).

Recordings were made from small DRG neurones (15–30 μm diameter) at a holding potential of −80 mV in the whole-cell, cell-attached and outside-out configurations with an L/M-EPC-7 amplifier (HEKA, Lambrecht, Germany); pipette resistances were 2–5 MΩ for whole-cell recordings and 5–15 MΩ for single-channel patches. After filtering at 3 kHz (−3 dB, 3 pole Bessel), data were acquired at a sample frequency of 1 kHz for whole-cell recordings and 20 kHz for single-channel recordings, and analyzed using pClamp 7 software (Axon Instruments, Union City, CA). Cells were superfused continuously (flow rate 0.5 ml/min) and a microprocessor-controlled application system with Peltier heat exchangers was used to apply fast temperature changes [10]. The temperature at

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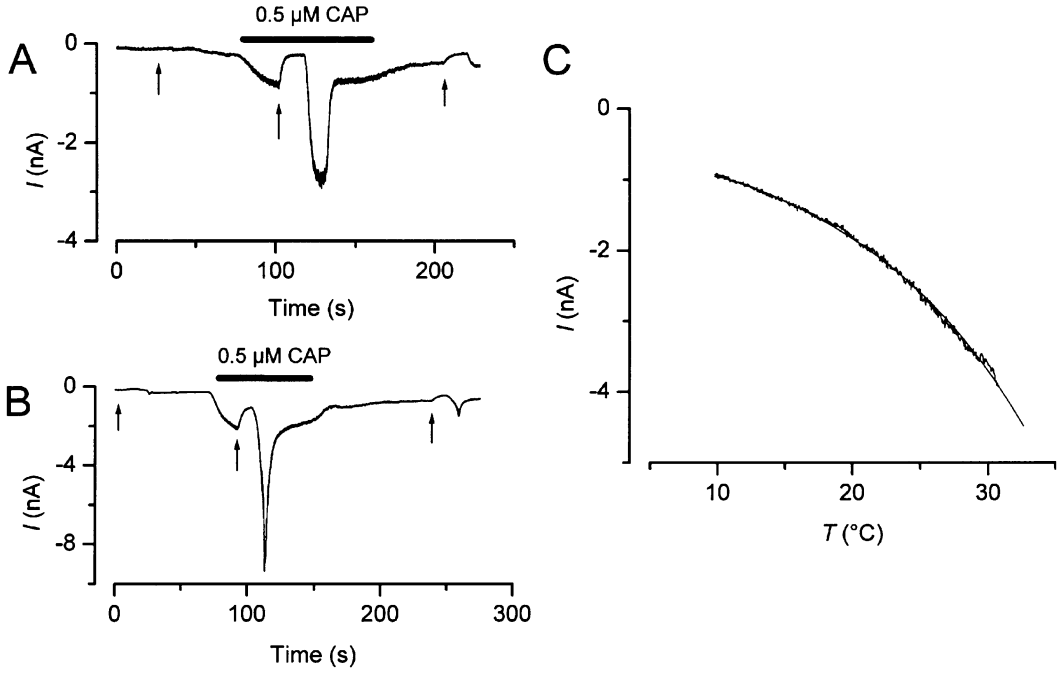

Fig. 1. Cooling inhibits capsaicin-induced currents in small DRG neurones. (A) Capsaicin (0.5 μM, bar) was applied at a base temperature of 30.4°C. Before, during and after capsaicin application, a cooling step to 13.9°C was applied (arrows). Warming to 37.7°C after the cooling step potentiated the capsaicin-induced current, as already described [14]. (B) Capsaicin (0.5 μM, bar) was applied at a base temperature of 22.5°C to another neurone. The following temperature protocol was applied (arrows): a cooling step to 10.7°C followed by a heating ramp to 37.6°C. (C) Temperature dependence of the capsaicin-induced current during the heating ramp in the experiment shown in (B) (with pre-capsaicin control current subtracted). This temperature dependence could be fitted with a monoexponential function which yielded a temperature coefficient (Q_{10}) of 2.1 in this experiment. Bath: Ca^{2+}-free extracellular solution; pipette: intracellular solution.

the cell during recordings was estimated after the experiments with the same temperature protocols using a miniature T-type thermocouple made of 50 μm wire (Physitemp, Clifton, NJ) located where the cell had been [11]. Normal extracellular solution contained (in mM): NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, and NaOH 4.54 (pH 7.4 at 25°C); the Ca^{2+}-free version was prepared by omitting $CaCl_2$ and increasing $MgCl_2$ to 3 mM. The isotonic KCl solution contained (in mM): KCl 144, $MgCl_2$ 3, HEPES 10, and KOH 4.54 (pH 7.4 at 25°C). The intracellular solution contained (in mM): CsCl 140, NaCl 10, EGTA 1, and HEPES 10 (adjusted to pH 7.2 at 25°C with NaOH). Capsaicin was applied at a concentration of 0.5 μM diluted freshly from a 1 mM stock solution in ethanol; it was applied only once to each coverslip. The same final concentration of ethanol (0.05%) was added to the control solution. All values are given as the mean ± SEM.

We used for analysis only cells which responded with a capsaicin-induced current larger than 200 pA. The amplitude of the capsaicin-induced current was 1.6 ± 0.5 nA (range 270 pA–5.98 nA) and the time to the peak of the current was 22.7 ± 1.7 s ($n = 13$).

In a first group of neurones ($n = 4$) the temperature at the cell was decreased from a base temperature of 31 ± 0.6 to 14.1 ± 0.6°C for 15–20 s (Fig. 1A). The same cooling step was applied before, during and after capsaicin application, and the control current before capsaicin application was subtracted from that in capsaicin. The capsaicin-induced whole-cell current at −80 mV was inhibited by 79.5 ± 8.9%, while the holding current before capsaicin was reduced by 30.5 ± 0.7%.

In a second group of cells ($n = 9$), the capsaicin-induced current was inhibited by 63.8 ± 3.2% and the holding current before capsaicin by 33.4 ± 3% when the cells were cooled from a base temperature of 22.7 ± 0.1 to 9.7 ± 0.3°C (Fig. 1B). In seven of these nine cells cooling steps were followed by heating ramps from 9.5 ± 0.3 to 29.8 ± 1.1°C before, during and after capsaicin application. The control current (i.e. during the first heat ramp before capsaicin application) was subtracted from the capsaicin-induced current, and the temperature coefficient (Q_{10}) of the resulting current was determined by exponential fitting (Fig. 1C; same experiment as Fig. 1B). The resulting Q_{10} was 2.3 ± 0.2 ($n = 7$, range 1.6–3.2).

In order to test whether the inhibition of the capsaicin-induced current by cooling is due to an effect on gating or only on ion permeation, we recorded single-channel activity induced by capsaicin in cell-attached and outside-out patches. The Q_{10} for the single-channel current was 1.5 between 10 and 25°C and 1.2 between 25 and 35°C (this effect is evident in Fig. 2A), which is in good agreement with values previously reported [7]. However, the open probability chan-

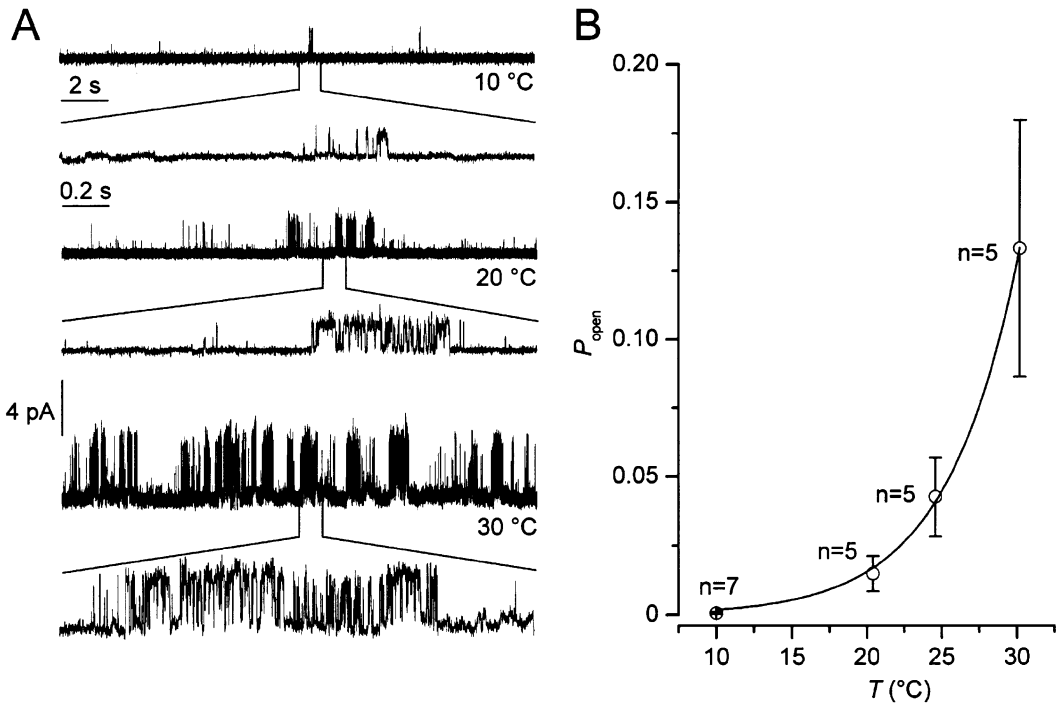

Fig. 2. Open probability of capsaicin-induced single-channel activity is strongly reduced by cooling. (A) Recordings of channel activity in the presence of 0.5 μM capsaicin in a cell-attached patch at three different temperatures. Sections of each recording are also shown on an expanded time scale. Bath: isotonic KCl solution; pipette: Ca^{2+}-free extracellular solution. The pipette potential was +80 mV relative to the bath. (B) Temperature dependence of P_{open} (see text) determined from several patches (numbers shown below each symbol; error bars show the SEM). The resulting Q_{10} from a monoexponential fit was 8.2.

ged substantially with temperature in this range. Fig. 2A shows capsaicin-induced single-channel activity at three different temperatures in a cell-attached patch while superfusing the cell with 0.5 μM capsaicin in isotonic KCl solution (pipette at +80 mV relative to the bath). In Fig. 2B we have plotted the temperature dependence of the open probability (P_{open}) of capsaicin-induced channel activity measured in seven patches containing one to five channels. We calculated P_{open} by measuring the fraction of time during which a given number of channels (none, 1, 2, 3, …) were open (to obtain the mean number of open channels), using a threshold of half the open channel amplitude, and then dividing by the number of active channels in the patch. The number of active channels in the patch was approximated by the maximal number of simultaneously open channels at the highest temperature (30°C, $n = 4$ and 35°C, $n = 3$) during a recording period of at least 20 s [6]. The single-channel data from which the P_{open} has been derived come from recordings in three different ionic conditions: outside-out recordings in Ca^{2+}-free extracellular solution at a holding potential of −80 mV ($n = 3$), cell-attached recordings in Ca^{2+}-free extracellular solution at a pipette potential of 0 mV (the effective membrane potential was the resting potential of the cell) ($n = 2$) and cell-attached recordings in isotonic KCl with a pipette potential of +80 mV ($n = 2$). These configurations yielded indistinguishable results and are pooled in Fig. 2B. Using an exponential fit, we calculated the temperature coefficient (Q_{10}) for the open probability of the capsaicin-induced single-channel activity as 8.2 between 10 and 30°C. The large standard errors are probably due to a degree of heterogeneity between individual patches (i.e. different temperature thresholds for different channels); for each individual patch the open probability increased monotonically with temperature between 10 and 30°C. The Q_{10} for the open probability of capsaicin-induced single-channel activity (8.2) is larger than that for the capsaicin-induced whole-cell currents (2.3). The reason for this difference may be the above mentioned heterogeneity of single-channel thresholds. It is unlikely to be due to a lack of intracellular modulation in excised patches, as results from excised patches and from cell-attached patches were indistinguishable.

As a conclusion, we have shown that cooling strongly inhibits capsaicin-induced currents in rat DRG neurones in primary culture, and that this inhibition is due mainly to a reduction in P_{open}. We have obtained a Q_{10} of 2.3 ± 0.2 for capsaicin-induced currents between 10 and 30°C, which is in good agreement with the value reported by Vyklicky et al. [14] of 2.1 for a different temperature range (23–52°C). These values of Q_{10} are much lower than those reported for the heat-induced current in DRG neurones (17.8; Ref. [14]), which may seem paradoxical since it is likely that the same molecule, VR1, is primarily responsible for both

currents [1,4]. However, it can be explained simply by proposing that capsaicin application reduces the activation energy for VR1 channel opening [14].

It is well known from clinical practice that local cooling can alleviate the pain associated with acute inflammatory states and injury. In human skin, injection of low pH buffers or topical capsaicin application induce pain which is abolished by cooling of the skin. Part of this effect is likely to involve spinal mechanisms [3], but peripheral mechanisms are clearly also important: ice cooling of the innervated skin area reduces the frequency of action potentials recorded in rat saphenous nerve upon intradermal injection of capsaicin [8]. Strong, but not mild, cooling also reduces inflammation after skin injury (for example, burns); these inflammatory symptoms are largely attributed to activation of VR1 [1,4]. Our finding that the current through VR1 is strongly inhibited by cooling provides an explanation for the peripheral analgesic and anti-inflammatory actions of strong cooling.

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