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Cold transduction by inhibition of a background potassium conductance in rat primary sensory neurones

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Abstract

Transduction in cutaneous cold receptors is poorly understood at present. We have studied this question using dorsal root ganglion (DRG) neurones in primary culture as a model of the otherwise inaccessible receptor terminal. Whole-cell recordings during cooling from 32 to 20°C revealed a large depolarization (>8mV) in 22 of 88 DRG neurones (25%), sometimes accompanied by action potentials. In cold-sensitive neurones cooling inhibited a time-independent background K^+ current (I_{cold}) which was resistant to tetraethylammonium and 4-aminopyridine. Ouabain elicited a substantially smaller depolarization than cooling, and no action potentials. We conclude that excitation by cooling in this model is primarily due to inhibition of I_{cold} and that the previously suggested role of the Na^+/K^+ adenosine triphosphatase is secondary. We suggest that I_{cold} may underlie cold transduction in cutaneous thermoreceptors.

Keywords: Cold; Thermoreceptor; Sensory transduction; Primary sensory neurone; Dorsal root ganglion; Background potassium current; Na^+/K^+ ATPase

Although rapid progress has been made in the last few decades in the understanding of some forms of sensory transduction, it is still not clear how skin cooling is detected, largely because of the inaccessibility of cutaneous thermoreceptor terminals. Present hypotheses are based on recordings of action potential activity from cold receptors in situ, and on analogy from cold effects on other neurone types; it is proposed that cold transduction depends on inhibition of the Na^+/K^+ adenosine triphosphatase (ATPase) and on differences in the temperature sensitivity of Na^+ and K^+ conductances [2,7,14]. These hypotheses have not been tested directly at the membrane level in cutaneous thermoreceptors until now. Here we have approached the question using whole cell patch clamp recording, with cold-sensitive dorsal root ganglion (DRG) neurones in short-term primary culture as a model of the receptor terminal.

Adult male Wistar rats (150–250 g) were killed with 100% CO_2. DRGs were dissociated with collagenase (0.6–1 mg/ml) and Dispase (3 mg/ml), 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 with 50 μg/ml gentamicin and either (a) N1 supplement [1] with 1 mg/ml bovine serum albumin or (b) 10% horse serum (all items from Sigma). To maximize the likely proportion of thermoreceptors we used only the L1-S1 ganglia, which innervate the scrotal area and the hind foot (known to be rich in thermoreceptors [5,11]), and added capsaicin (2 μM; Fluka, Buchs, Switzerland) throughout the time in culture to kill capsaicin-sensitive nociceptors [16]. Whole-cell and amphotericin perforated patch recordings were made from small DRG neurones with an EPC-7 amplifier (HEKA, Lambrecht, Germany); pipette resistances were 1.5–3 MΩ and seals 2–10 GΩ. Data were analyzed using programs written in IDL (Research Systems, Boulder, CO) and all values are given as mean ± SEM. Neurones with a resting potential more positive than −40 mV were excluded from further analysis.

Cells were adapted at 32°C for 5–10 min before recording, and cooling stimuli (to 20°C at the cell) were applied locally: solutions were passed through a chamber (15 × 30 mm, vol. ~100 μl) made from two glass coverslips separated by a polystyrene spacer, sandwiched between Peltier elements whose current was regulated by a microprocessor-

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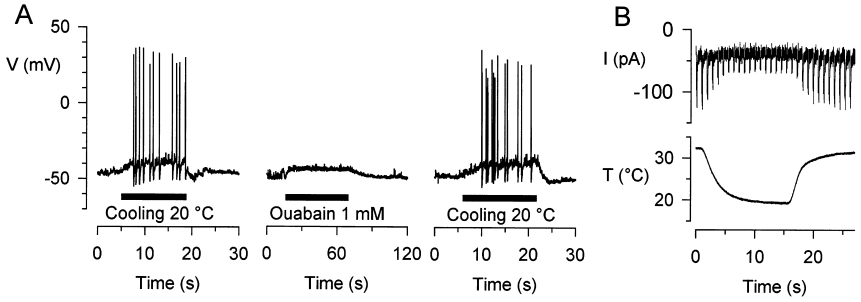

Fig. 1. (A) Cooling depolarizes some DRG neurones, and its effects cannot be accounted for by inhibition of the Na^+/K^+ ATPase. Left: cooling-induced depolarization and action potentials. Temperature at the cell is shown (see (B) for the time course); solution left the Peltier device at 12°C. Centre: in the same cell, 1 mM ouabain depolarized to a much smaller degree than cooling (~50%) without inducing action potentials. Right: ~2 min later, the cell responded again to cooling as before. (B) Cooling reduces the resting conductance in cold-sensitive DRG neurones. Above: current during cooling in standard extracellular solution at a holding potential of −80 mV; pulses to −100 mV (150 ms duration, 750 ms interpulse interval) were applied to measure input resistance. Below: the cooling stimulus, measured at the cell (see text).

based feedback controller to control the chamber temperature. Flow rate was ~1.5 ml/min, and the exit tubing (length ~2 cm, inner diameter 1 mm) was placed 1–2 mm from the cell. The temperature at the cell during recordings was estimated after the experiments by repeating the thermal stimuli, with a miniature type T thermocouple (Physitemp, Clifton, NJ) placed where the cell had been. Normal extracellular solution contained (in mM): NaCl 140, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, NaOH 4.55 (pH 7.4 at 25°C); the Ca-free version (see Fig. 2) was prepared by simply omitting $CaCl_2$. The pipette solution contained: K_2SO_4 60, KCl 35, NaCl 10, $MgCl_2$ 1, sucrose 40, HEPES 10, EGTA 1, NaOH 3.45, KOH 2.35 (pH 7.2 at 25° C; this was supplemented with 2 mM MgATP and 0.1 mM LiGTP for whole-cell recording). High-K^+, Ca-free extracellular solution contained: KCl 144, $MgCl_2$ 1, HEPES 10, KOH 4.55 (pH 7.4 at 25°C).

A population of DRG neurones (22 of 88 neurones, 25%) responded to cooling with a depolarization of 8–20 mV, accompanied in five neurones by action potentials (Fig. 1A); this response was reproducible within each cell (2–6 times over a total period of up to 80 min). The remainder (66/88, 75%) were depolarized by ≤5 mV (not shown). Response amplitudes were bimodally distributed (not shown), and we defined the two groups as cold-sensitive and cold-insensitive, respectively. The proportion of DRG neurones responding to cooling is consistent with the numbers of cold receptors found in vivo [11]. The two groups did not differ in cell size, initial resting potential or action potential duration (Table 1), although more cold-sensitive than cold-insensitive cells showed an inflection during the repolarization phase of the action potential. Cold-responsive cells were found in both the dark and bright cell populations, suggesting that we were recording from the somas of both Aδ- and C-fibres [10]; cold receptors in the rat fall into both groups [11]. To exclude possible pH effects (the pH of a HEPES-buffered solution rises by nearly 0.2 on cooling by 12°C) we applied extracellular solution at pH 7.65 to five cold-sensitive neurones at 32°C; this had no effect on membrane potential, implying that the neurones had been responding to cooling and not simply to altered pH.

Inhibition of the Na^+/K^+ ATPase is thought to play a role in cold transduction [2,14]. We tested this by applying 1 mM ouabain, which is expected to block the Na^+/K^+ ATPase in DRG neurones by 95% [3]. In five cold-sensitive neurones, 1 mM ouabain elicited a depolarization of 10–50% of that induced by cooling, and never evoked action

Table 1
Properties of cold-sensitive and cold-insensitive DRG neurones[a]

	Cold-sensitive (*n*)	Cold-insensitive (*n*)	*P*
Diameter (μm)	23.6 ± 1.1 (22)	24.8 ± 0.6 (64)	0.33
Initial resting potential (mV)	−56.2 ± 2.0 (22)	−54.0 ± 1.0 (66)	0.30
Action potential duration (ms)	5.9 ± 1.0 (9)	5.6 ± 0.4 (27)	0.67
Inflection during repolarization	7/9 (78%)	13/27 (48%)	
Input resistance at 32°C (MΩ)	216 ± 62 (5)	326 ± 41 (12)	0.21
Increase in input resistance at 20°C (%)	168 ± 47 (5)	92.5 ± 8 (12)	0.015*

[a] All values are given as mean ± SEM. Diameter was measured as the mean of the largest and smallest diameter and action potential duration at 90% repolarization. All *P*-values are based on Student's *t*-test, either two-tailed or one-tailed (asterisk).

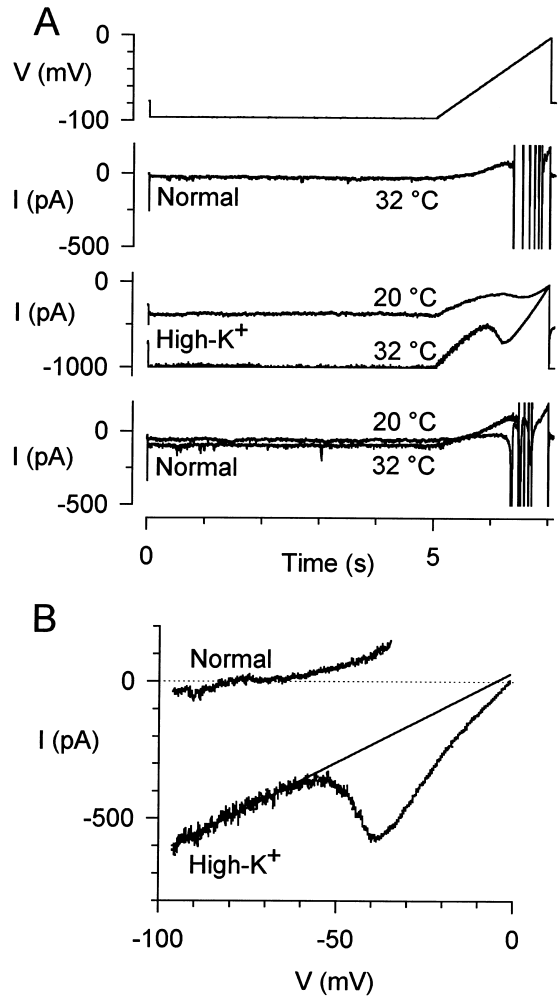

Fig. 2. (A) Currents during voltage ramps at 32 and 20°C in normal and high-K^+ extracellular solution. The top trace shows the applied voltage. Currents were recorded in (top to bottom): Ca-free normal extracellular solution at 32°C, high-K^+ extracellular solution at 20 and 32°C, and again in Ca-free normal extracellular solution at 20 and 32°C. Here all solutions contained 10 mM TEA and 1 mM 4-AP; similar results were obtained without TEA or 4-AP. The cell had extensive neurites and unclamped action potentials are visible. (B) The cold-sensitive K^+ current I_{cold}, derived by subtraction from the recordings above. I_{cold} in Ca-free normal extracellular solution reverses between −80 and −70 mV. The reversal potential of I_{cold} in high-K^+, Ca-free extracellular solution, estimated from a regression line fitted to the region from −100 to −60 mV (straight line), is close to 0 mV.

potentials (Fig. 1A, centre). We conclude that inhibition of the Na^+/K^+ ATPase is not the only mechanism by which cooling excites cultured DRG neurones, and is probably not the major one.

If an ionic conductance is also involved in cold-induced depolarization, cooling could either activate an inward current or inhibit an outward current. To distinguish these we measured membrane conductance during cooling, using brief pulses to −100 mV from a holding potential of −80 mV (Fig. 1B). The input resistance at 32°C of cold-sensitive and cold-insensitive neurones did not differ (Table 1). Cooling increased the input resistance of all neurones, indicating that it inhibits an outward current active at the resting potential rather than activating an inward current. If this current is important for cold-induced depolarization, one would expect a greater increase in input resistance in cold-sensitive than in cold-insensitive neurones, and this was indeed found to be the case (Table 1).

We therefore hypothesized that a major component of the resting conductance of cold-sensitive DRG neurones is a cold-sensitive potassium current. To study this current in the region of the resting potential, we recorded in a high-K^+, Ca^{2+}-free extracellular solution, using voltage ramps from −100 to 0 mV at 32 and 20°C. In cold-sensitive DRG neurones, the current at −100 mV in high-K^+ solution was reduced by 49–72% on cooling from 32 to 20°C (61.1 ± 2.7%, $n = 8$; Fig. 2A). Current during voltage ramps at both temperatures was linear between −100 and −60 mV; when the cold-sensitive current was isolated by subtraction, a regression line fitted to this linear region gave an extrapolated reversal potential close to 0 mV (Fig. 2B). In Ca-free normal extracellular solution the cold-sensitive current was small and reversed between −75 and −60 mV (−69.1 ± 1.9 mV, $n = 8$; Fig. 2B). This indicates that a cold-sensitive potassium conductance (which we have termed I_{cold}) forms the greater part of the resting conductance in cold-sensitive DRG neurones.

Using small voltage pulses around the −80 mV holding potential we found that I_{cold} was time-independent (not shown). It was rather insensitive to tetraethylammonium (TEA) and 4-aminopyridine (4-AP), with cold-sensitive current at −80 mV being reduced by 16.5 ± 6.8% by 10 mM TEA ($n = 4$) and 41 ± 13% by a mixture of 10 mM TEA and 1 mM 4-AP ($n = 2$). We suspected that the TEA-insensitive background 'flicker' K^+ channel found in small myelinated axons [8] might underlie I_{cold}, and tested this in two cold-sensitive neurones with bupivacaine, a potent blocker of the flicker channel ($IC_{50} = 165$ nM); 10 μM bupivacaine did not alter cold-sensitive current, indicating that I_{cold} cannot be attributed to this channel.

To our knowledge, this is the first study of the changes in membrane potential and the ionic currents involved in cold transduction in peripheral thermosensitive neurones. We have shown that a population of DRG neurones is sensitive to cold, but that cold transduction in these neurones cannot be accounted for by inhibition of the Na^+/K^+ ATPase, as previously suggested for cold receptors in vivo [2,14]; the primary cause of the depolarization and increase in excitability induced by cooling appears to be the inhibition of a background K^+ current active at the resting potential. This current, which we have termed I_{cold}, is highly sensitive to temperature in the range between about 20 and 30°C where mammalian cold receptors show maximal activity [6,14], and we suggest that it may be involved in cold transduction in vivo.

We can set an approximate upper limit on the contribu-

tion of non-specific effects, (e.g. cold-induced changes in seal and membrane leak) to our measurements. Based on the reversal potential of the cold-sensitive current (~ -70 mV) and the K^+ equilibrium potential (-94 mV), a leak reversing at 0 mV could contribute a maximum of 25% of the cold-sensitive current in normal extracellular solution (if I_{cold} is perfectly selective for K^+; if not, the estimated leak would be smaller). The resting conductance was ~3–5 nS (see Table 1), suggesting a leak of not more than ~1 nS, and the conductance carrying I_{cold} in high-K^+ solution was about 5–10 nS at 32°C (Fig. 2B); so our measurements reflect primarily cold-induced changes in ionic current and not in leak. The resting ionic conductance is presumably heterogeneous and only partly due to I_{cold}: using the DRG soma as a model means that I_{cold} will be expressed along with the mixture of other K^+ channels normally found in the soma, some of which are active at rest [15]. Both this and the leak would dilute the effect of I_{cold} on our measurements, meaning that its true temperature sensitivity is probably higher than the overall Q_{10} of 2–4 that we report here. The Q_{10} of ion permeation is generally low (1.2–1.3 [4]), suggesting either that temperature is acting on the gating of the channel underlying I_{cold} or that its permeation is unusually temperature-sensitive.

Inhibition of a background K^+ current is an unusual mechanism of sensory transduction – we are not aware of any other modalities where it has been encountered – but it is a common mechanism of neuromodulation. In this context, attention has recently focused on the two-pore-domain K^+ channels, and functions are rapidly being found for members of this family (see Ref. [13] and references therein). Very recently two of them have been shown to produce heat-activated K^+ currents, mouse TREK-1 [12] and TWK-18 from *Caenorhabditis elegans* [9]; the former was suggested to play a role in temperature sensing [12]. I_{cold} is, as far as we are aware, the first native heat-activated background K^+ current to have been described; although no firm conclusions can be drawn at the moment about its molecular nature, its characteristics (activity at the resting potential, time-independence, resistance to TEA and 4-AP) suggest that it could also be a member of the two-pore-domain K^+ channel family.

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