Physiology

Cold current in thermoreceptive neurons

We sense the temperature of our skin and surroundings using specific thermoreceptors, which are sensitive to cold and warmth[1], but little is known about how these receptors transduce temperature into electrical activity. We have discovered an inward ionic current that is activated by moderate cooling in a small number of rat sensory neurons. This current has features that are found in intact cold receptors, including sensitization by menthol, adaptation upon sustained cooling, and modulation by calcium, and is likely to be important in cold sensing.

Cutaneous receptors are difficult to study, as they are small and inaccessible in the skin, so cultured dorsal root ganglion (DRG) neurons are widely used as a model system because they express membrane proteins that would normally be present at their receptor termini. We found previously that very few DRG neurons generate action potentials in response to cold[2], so we measured the intracellular calcium-ion concentration by imaging[3] to preselect cold-responsive rat DRG neurons after 2–4 days in primary culture, applying thermal stimuli with a Peltier-based device[4] from a base temperature of 32 °C. Of 643 DRG neurons, 45 (7%) responded to cooling to 20 °C by increasing their intracellular Ca^{2+} concentrations. This is consistent with the proportion of cold thermoreceptor afferents in the rat hindlimb[5,6].

In the cold-responsive DRG neurons, we measured membrane potential and ionic currents using perforated-patch recording. We raised the temperature (for about 10 s) from 32 °C to 37 °C, and then applied a ramp from 37 °C to 18–20 °C over a 30-s period. From a resting potential of -53.3 ± 7.9 mV (s.d.; $n=32$), the cooling ramp induced a depolarization of 7–49 mV (22.3 ± 11.2 mV) and high-frequency action potentials (Fig. 1a). Cooling at -80 mV elicited an inward current (Fig. 1b) with a threshold of 23–34 °C (28.7 ± 2.7 °C; $n=27$) and a maximum amplitude of 40–350 pA.

This current was present in all cold-responsive neurons tested, and was absent from all of 16 unresponsive ones, showing that it is not a ubiquitous current in primary somatosensory neurons. By applying temperature steps, we found that the activation and deactivation of this current were as rapid as (and perhaps limited by) the time course of the thermal stimulus, which has a time constant of about 5 s (ref. 4). During sustained cold stimulation, the current adapted almost completely, with a time constant of 62–69 s ($n=4$; Fig. 1), consistent with the slow adaptation of cold receptors *in vivo*[7].

The cold-receptor stimulant (−)menthol[8,9] substantially increased the amplitude of the cold-induced current and shifted its activation threshold towards higher temperatures, by 4.2 ± 2.0 °C at 10 µM ($n=16$) and by 7.6 ± 2.6 °C at 100 µM ($n=9$; Fig. 1b). A low extracellular Ca^{2+} concentration potentiated the current (1.5–2.8-fold at 0.1 mM; $n=6$), whereas increasing the extracellular Ca^{2+} concentration to 10 mM reduced it slightly and reversed the sensitization induced by 10 µM menthol ($n=4$); intact cold receptors are similarly affected by changes in Ca^{2+} concentration[9,10]. All of the effects due to menthol and altered Ca^{2+} were reversible.

The reversal potential of the cold- and menthol-induced current obtained by subtraction during voltage ramps was $+13.3\pm5.2$ mV ($n=6$), indicating that it is probably a mixed-cation current. We cannot assign it to one of the known families of cation channels involved in somatosensory transduction, as amiloride (100 µM), which blocks acid-sensitive and mechanosensitive channels of the degenerin family, had no effect ($n=6$), whereas ruthenium red (10 µM), a blocker of the heat-sensitive VR-1 and VRL-1 channels, reversibly increased the current ($n=6$).

Our observations suggest that the mode of action of menthol on cold receptors should be reconsidered. An early model indicated that menthol stimulates cold receptors by blocking voltage-dependent Ca^{2+} channels, leading to a reduction in intracellular Ca^{2+} and inhibition of Ca^{2+}-dependent K^+ channels[9]. However, we and others[11] have since discovered that menthol stimulates entry of Ca^{2+} and increases intracellular Ca^{2+} concentration in cold-sensitive neurons; the stimulation of cold receptors by menthol can be explained more simply by sensitization of the cold-induced inward Ca^{2+} current.

To our knowledge, this is the first description of an ionic current that is activated by cooling. The properties of this current can account for several features of cold-receptor function: it is activated over the temperature range in which mammalian cold receptors are most sensitive[1]; its rate of adaptation is similar to that observed *in vivo*[7]; and its potentiation by menthol and modulation by calcium are similar to the response of intact cold receptors[8–10]. We propose that this cold-activated current is the principal determinant of cold-receptor activity, and that inhibition of a background K^+ current[2] and of the electrogenic Na^+/K^+-ATPase[12] are of secondary importance.

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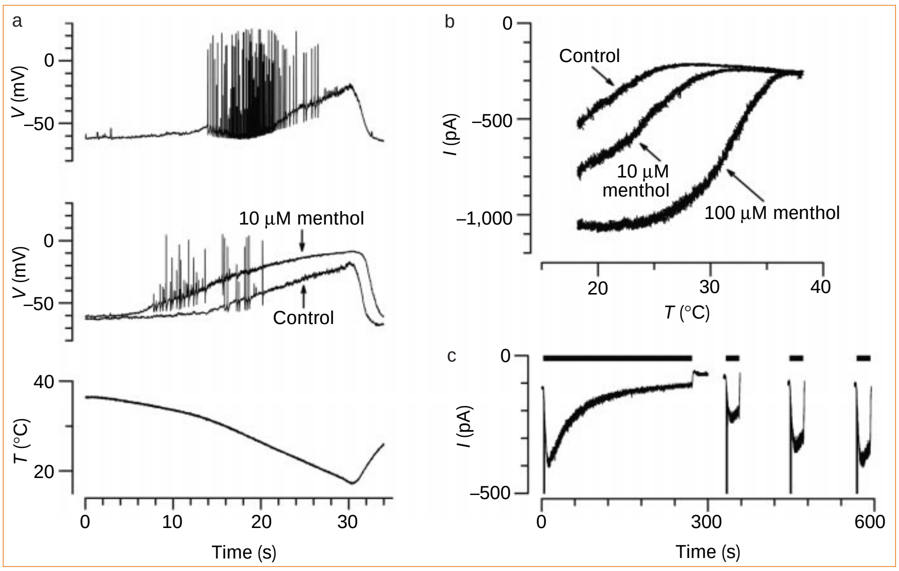

Figure 1 Cold sensitivity and cold-induced inward current in primary sensory neurons. **a,** Top, depolarization and action potentials during cooling in a rat dorsal root ganglion neuron; middle, sensitization by 10 µM (−)menthol in the same neuron (10 µM bupivacaine was added to reduce action-potential frequency); bottom, thermal stimulus. **b,** Current–temperature relationship of cold-induced current during ramps similar to those in **a**, and the sensitization induced by 10 µM and 100 µM (−)menthol. **c,** Adaptation of the cold-induced current during prolonged cooling to 15 °C (horizontal bars) and recovery at 32 °C; action potentials in cell processes are apparent.

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