Coexpression of heat-evoked and capsaicin-evoked inward currents in acutely dissociated rat dorsal root ganglion neurons

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Abstract

Noxious heat is able to activate heat-sensitive nociceptors in the skin very rapidly, but little is known about the mechanisms by which heat is transduced. We used the whole-cell patch-clamp technique to study the effects of noxious heat and capsaicin on freshly dissociated rat dorsal root ganglion neurons in vitro. Using temperatures between 41 °C and 53 °C, 8 of 19 small neurons (ø ≤ 30 μm) exhibited a heat-evoked inward current. All heat-sensitive neurons tested were also capsaicin-sensitive. Moreover, the heat response tended to be enhanced after capsaicin (360 ± 150 pA versus 125 ± 45 pA, P < 0.1, n = 7). Two of five heat-insensitive neurons were excited by capsaicin; both neurons developed a heat response after capsaicin. Large neurons (ø > 30 μm) did not respond to heat (0/7), and were not sensitive to capsaicin either. These findings indicate that heat stimuli may directly activate capsaicin-sensitive primary nociceptive afferents. © 1997 Elsevier Science Ireland Ltd.

Keywords: Heat pain; Patch-clamp; Primary sensory neurons; Capsaicin

Polymodal C-fiber nociceptors in the skin of primates and humans respond within a few milliseconds to rapid temperature increases generated by infrared lasers [4,13]. The short latencies observed in vivo suggest that the peripheral axon terminals of small dorsal root ganglion (DRG) neurons possess a direct transduction mechanism for heat stimuli. The first direct confirmation of such a mechanism was provided by recordings of heat-evoked action potentials in cultured trigeminal ganglion neurons [1]. A recent study in DRG neurons of neonatal rats that had been in primary culture for a few days demonstrated that heating the cell soma to a temperature of 49°C for about 1 s induced inward currents in some of the neurons [5].

In contrast to the peripheral axon terminals, the soma of DRG neurons is accessible to patch-clamp recordings and is considered to be a model for its own peripheral terminals [15]. Criteria to identify the somata of nociceptive DRG neurons include small cell size, action potential shape, and excitability by capsaicin [6]. The aim of this study was to determine how many acutely dissociated DRG neurons of adult rats are heat-sensitive, and to test for coexpression with capsaicin sensitivity. A preliminary report of these data has been given in abstract form [8].

Adult Sprague–Dawley rats (130–250 g) were anesthetized with diethylether, decapitated, and 10–15 DRGs were dissected quickly. The cells were dissociated with collagenase CLS II (5–10 mg/ml, 30–45 min; Biochrome) and trypsin (0–1 mg/ml, 10–12 min; T-8003, Sigma) dissolved in F12-Dulbecco’s modified Eagle’s medium (D-8900, Sigma) at 37 °C. Cells were plated on culture dishes and were stored in a humidified 5% CO 2 atmosphere at 37°C before being used for electrophysiological recordings 2–30 h after dissociation.

The patch-clamp recordings were performed in the whole-cell mode at a holding potential of −80 mV in F12 medium (pH 7.4) with borosilicate micropipettes (tip resistance 5–6 MD) that were filled with (in mM) 160 KCl, 8.13 EGTA, and 10 HEPES. We used the Axopatch 200A amplifier and the pCLAMP6 software for offline analysis (Axon Instruments).

After measuring the cell diameter and the cross sectional
area the neurons’ excitability was tested with depolarizing voltage pulses (prehyperpolarization to −100 mV) from −90 to +100 mV in 10 mV steps for 51 ms, by which a biphasic current with a fast inward and a prolonged outward current could be elicited. The reversal potential of the fast inward current was close to the equilibrium potential of sodium. The resting membrane potential, and the membrane capacity were measured.

The heat-evoked currents were determined qualitatively by application of about 50 μl of heated extracellular solution through a Pasteur pipette fixed on a micromanipulator (n = 26). Measurements with a fast temperature sensor (Physitemp BAT-12, τ = 5 ms) revealed peak temperatures of 41°C, 49°C, and 53°C for the three preheating temperatures used. These stimuli were repeated 1–5 times with 15 s intervals, and the responses were averaged afterwards. Subsequently the same amount of extracellular solution at room temperature (23°C) was applied 1–3 times as a control (n = 15). The capsaicin sensitivity was determined qualitatively by superfusion with 1 μM capsaicin at room temperature. The heat-evoked current (121 ± 41 pA) was significantly larger than control (26 ± 14 pA, P < 0.05, paired Wilcoxon test, n = 13). Open circles show heat-evoked currents without control. Heat-sensitivity was only considered when the current exceeded the largest response to control (150 pA). (B) Large neurons (ø ≥ 30 μm, n = 7). The measured current under heat was 21 ± 15 pA. No cell fulfilled the criteria of heat sensitivity. (C) Stacked bar graph of heat-sensitive (filled bars) and insensitive neurons (open bars) as a function of diameter (n = 26). All heat-sensitive cells were not larger than 30 μm. (D) Stacked bar graph of capsaicin-sensitive (filled bars) and insensitive neurons (open bars) as a function of diameter (n = 11). All capsaicin-sensitive cells were not larger than 30 μm.

All capsaicin-sensitive neurons had a diameter ≤30 μm (area ≤700 μm²). Fig. 2B shows that none of the seven large neurons (≥30 μm) exhibited any heat sensitivity. The mean current in large neurons following heat (21 ± 15 pA) was not different from that in small neurons following room temperature (17 ± 13 pA).

The reproducibility of the heat response was tested in 22 neurons including seven heat-sensitive and 15 heat-insensitive neurons (11 small, four large). The currents evoked by the first and second application were highly correlated (Spearman correlation coefficient rₚ = 0.92, P < 0.01) and not significantly different (100 ± 40 pA versus 145 ± 80 pA).

Capsaicin sensitivity was tested in 11 neurons. Fig. 2D shows the population response of the capsaicin-sensitive neurons as a function of their diameters. Similar to heat currents were found in 8/26 neurons (Fig. 2C). All heat-sensitive neurons had a diameter ≤30 μm (area ≤700 μm²).
neurons were not sensitive to capsaicin or heat (0/2). Either, could be activated by heat after capsaicin. Large that was not initially sensitive to heat and capsaicin neurons were activated by capsaicin, and these two neurons cin-sensitive (4/4). Two of the five small heat-insensitive P larger after capsaicin (360 \& 150 pA versus 125 ± 45 pA, P < 0.1, paired Wilcoxon test, n = 7).

In eight cells heat sensitivity was also tested after capsai- In five of seven small neurons capsaicin enlarged the cin. In five of seven small neurons capsaicin enlarged the heat response. An example of an enhanced inward current is shown in Fig. 3A (before capsaicin) and Fig. 3B (after capsaicin). Fig. 3C summarizes the heat responses of all small neurons tested. The mean current of all neurons was shown in Fig. 3). In our study nearly 50% of the small neurons, but none of the large neurons turned out to be heat-sensitive. In neonatal rats, Cesare and McNaughton [5] found 56% neurons responsive to 49°C, using a preparation of small neurons only (ø < 25 mm). In the rat skin 55% of the C-fibers in vitro and 40% of the C-fibers in vivo are sensitive to heat stimuli [10]. In contrast to a previous study that reported heat sensitivity of small DRG neurons after 4-6 days in culture [5], we were able to demonstrate heat-evoked inward currents as early as 4 h after dissociation. Our results indicate that heat sensitivity may be constitutively expressed in the soma membrane. Alternatively, heat sensitivity of the soma may be a consequence of axotomy [3]. The time after dissociation may be long enough; the A-fiber mechanosensitivity of a neuroma begins 4 h after axotomy [9] and increases thereafter due to axonal transport. It is known that capsaicin sensitivity is present immediately in acutely isolated DRG cells whereas the sensitivity to bradykinin is developed during a few days in culture [6,14]. Thus the expression of heat sensitivity is similar to capsaicin, but different from bradykinin.

We have demonstrated that heat and capsaicin sensitivity are coexpressed in DRG neurons and that capsaicin is able to enhance heat-evoked inward currents. Coexpression of heat and capsaicin sensitivity of primary afferents in vitro have been demonstrated in rat [12] and in monkey [2]. Heat sensitization by capsaicin, however, is only observed at low doses [2], whereas at higher doses capsaicin causes a blockade [7]. The type of capsaicin-evoked currents described in this paper is most likely the slower component of capsaicin-activated currents according to the time course, the low concentration due to the dilution in the bath, and the relatively small peak current [11].

In summary, we have demonstrated heat-evoked inward currents in about half of the small DRG neurons, but in none of the large neurons. All heat-sensitive DRG neurons responded with an inward current to capsaicin, and the heat response was enlarged by preceding capsaicin application. These findings confirm the correlation of capsaicin-sensitivity and heat-sensitivity of primary afferent neurons reported from in vivo studies.

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