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Research article

Mechanical sensitivity and electrophysiological properties of acutely dissociated dorsal root ganglion neurons of rats

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HIGHLIGHTS

- Primary afferent fibers use mechanically activated (MA) currents to transduce innocuous and noxious mechanical stimuli.
- It is largely unknown about the differences in MA currents between the afferents for sensing innocuous and noxious stimuli.
- This study identified differences in MA currents between non-nociceptive-like and nociceptive-like DRG neurons.
- We also showed differences in electrophysiological properties between these two groups of mechanically sensitive neurons.
- Our findings provide insights into mechanisms of sensory encoding for innocuous and noxious mechanical stimuli.

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ABSTRACT

Primary afferent fibers use mechanically activated (MA) currents to transduce innocuous and noxious mechanical stimuli. However, it is largely unknown about the differences in MA currents between the afferents for sensing innocuous and noxious stimuli. In the present study, we used dorsal root ganglion (DRG) neurons acutely dissociated from rats and studied their MA currents and also their intrinsic membrane properties. Recorded from small-sized DRG neurons, we found that most of these neurons were mechanically sensitive (MS) showing MA currents. The MS neurons could be classified into nociceptive-like mechanically sensitive (Noci-MS) and non-nociceptive-like mechanically sensitive (nonNoci-MS) neurons based on their action potential shapes. Noci-MS neurons responded to mechanical stimulation with three types of MA currents, rapidly adapting (RA), intermediately adapting (IA), and slowly adapting (SA) currents. In contrast, almost all nonNoci-MS neurons showed RA current type in response to mechanical stimulation. Mechanical thresholds had a broad range for both nonNoci-MS and Noci-MS neurons, and the thresholds were not significantly different between them. However, MA current densities were significantly smaller in Noci-MS than in nonNoci-MS neurons. Noci-MS and nonNoci-MS neurons also showed significant differences in their electrophysiological properties including action potential (AP) thresholds and AP firing patterns. These differences may contribute to the differential sensory encoding for innocuous and noxious mechanical stimuli.

1. Introduction

Many primary afferent endings in the skin and other tissues detect mechanical stimuli such as gentle touch, stretch, and noxious pinch. This is important for sensory tasks including social interaction, environment exploration and avoiding harmful stimuli. The first step toward achieving these tasks is mechanotransduction that conveys mechanical stimuli into electrical signals. Mechanotransduction is found to be mainly mediated by mechanically activated (MA) ion channels in primary afferent neurons [1–5], and these ion channels largely contribute to the mechanical sensitivity of primary afferent fibers.

Mechanically sensitive (MS) afferent neurons have been observed in dorsal root ganglion (DRG) neurons in culture, and these neurons respond to membrane displacements with MA currents. MA currents in DRG neurons display a wide range of kinetics from rapidly adapting (RA), intermediately adapting (IA), to slowly adapting (SA) [2,6]. RA currents in DRG neurons have decay time constants of less than 10 ms [2,3,6]. The molecular identity conferring RA currents in DRG neurons has recently been elucidated to

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be Piezo2, a large transmembrane protein that forms MA channels in mammalian primary afferent neurons [6]. Piezo2 channels are also expressed on Merkel cells of hair follicles and touch domes of the skin, where they are key molecules for the transduction of gentle touch [7–9]. The molecular identities mediating IA currents and SA currents in DRG neurons are not fully understood, but a recent study has identified tentonin 3 as an ion channel mediating MA currents with slow kinetics in a subpopulation of DRG neurons with proprioceptive function [10]. Although the role of primary afferent Piezo2 channels in gentle touch has recently been demonstrated using conditioning Piezo2 knockout mice, their potential role in mechanical nociception remains to be determined [11].

Most of previous studies have used cultured DRG neurons to study sensory functions of MA channels [1,3,6]. It has been shown with cultured DRG neurons that many capsaicin-sensitive neurons responded to mechanical stimulation with MA currents displaying either RA or SA current kinetics (Drew et al., 2002). This finding may suggest a potential role of MA currents in mechanical nociception. However, DRG neurons grown in culture change their functional phenotypes by altering their nociceptive markers, transducer expression, and intrinsic electrophysiological properties. MA currents have also been found to be significantly affected by nerve growth factors that are commonly used for culturing DRG neurons [12]. These changes due to culture conditions may have obscured functions of MA channels in terms of their roles in mechanical transduction for non-nociceptive and nociceptive neurons. In the present study, to minimize the potential cell phenotype changes we used neurons acutely dissociated from rat DRGs and characterized their mechanical sensitivity and electrophysiological properties.

2. Materials and methods

Sprague Dawley rats aged 5–9 weeks were used. Animal care and use conformed to NIH guidelines for care and use of experimental animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham. Acutely dissociated DRG neurons were prepared as described in our previous work [13]. In brief, rats were deeply anesthetized with isoflurane and sacrificed by decapitation. Bilateral DRGs were dissected out and incubated with dispase II (5 mg/ml) and type I collagenase (2 mg/ml) in 2 ml bath solution at 35 °C for 45 min. The bath solution was the same one used for cell perfusion in electrophysiology experiments (see below). After a rinse, DRGs were triturated to dissociate the neurons in the bath solution, and the dissociated cells were plated on glass coverslips coated with poly-D-lysine and maintained at room temperature. Whole-cell patch-clamp recordings were performed within 1–4 h after cell plating.

The cells were perfused with normal bath solution flowing at 1 ml/min in a 0.5 ml chamber on the stage of an Olympus IX70 microscope. The bath solution contained (in mM) 150 NaCl, 5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 10 glucose, and 10 HEPES, pH of 7.4, osmolarity of 320 mosM. The internal solution of electrodes contained (in mM) 135 K-gluconate, 0.5 $CaCl_2$, 2 $MgCl_2$, 5 KCl, 5 EGTA, 10 HEPES, 2 Na_2-ATP, 0.5 Na_2-GTP, pH of 7.2 and osmolarity of 315–325 mOsm. Recording electrode resistance was ~5 MΩ. The junction potential was −15 mV and was adjusted. The series resistance of each recording was below 30 MΩ and was not compensated. Recording signals were amplified with Axopatch 200 B (Axon Instruments), filtered at 2 kHz, and sampled at 5 kHz using pCLAMP 10 (Axon Instruments). Unless otherwise indicated, all reagents were purchased from Sigma.

To determine MA currents, DRG neurons were held at −75 mV and mechanical stimulation was applied to DRG cell bodies using a heat-polished glass pipette as a probe (tip diameter approximately 4 μm). The probe was controlled by a piezo-electric device (Physik Instruments, Auburn, MA). The probe was positioned at an angle of 45° to the surface of the dish. The tip of the probe and the recorded cell were visualized as live images on a monitor. The live images were captured continuously through a CCD camera that was connected to the microscope fitted with a 40x objective. The tip of the mechanical probe was positioned so that a 1 μm movement contacted the cell and was considered as a 1-μm membrane displacement. The probe was moved at a speed of 0.5 μm/ms. Unless otherwise indicated, stepwise membrane displacements were produced by the mechanical probe for up to 15 μm in the increment of 1 μm each step and the duration of each step was 500 ms. To determine membrane and AP properties of recorded DRG neurons, under the whole-cell current-clamp mode, step current pulses were injected into cells through patch-clamp electrodes. The step currents were applied from −50 pA to 1500 pA in an increment of 25 pA per step and the duration of each pulse was 1 s. All recordings were performed at the room temperature of 24 °C.

Recording data were analyzed using Clampfit 10 software. Data are reported as mean ± SEM. Statistical significance ($^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$) was assessed by two-way ANOVA with post-hoc Fisher's LSD test.

3. Results

To enhance the stability of acutely dissociated DRG neurons in recording chambers during patch-clamp recordings of mechanically activated (MA) currents, we used a glass pipette tip as an anchor point to restrict cell movement during membrane displacements (Fig. 1A). This modification helped improve the reproducibility of MA currents recorded from acutely dissociated DRG neurons. All recorded neurons were small-sized cells with diameters ≤ 35 μm. Membrane displacement could evoke inward MA currents under the voltage-clamp mode (Fig. 1B) and action potential firing under the current-clamp mode (Fig. 1C). With membrane displacements up to 15 μm, 82% (n = 73) of DRG neurons were mechanically sensitive (MS) showing MA currents (Fig. 1D), and the remaining 18% (n = 16) cells did not show any detectable MA currents and were considered as mechanically insensitive (MI) cells (Fig. 1D). Action potential (AP) shapes have been used as indicators of nociceptive-like (Noci) and non-nociceptive-like (nonNoci) DRG neurons, and a Noci neuron usually has a broad AP with a shoulder during repolarization but a nonNoci neuron has a narrow AP without a repolarization shoulder [14]. Accordingly, we classified our DRG neurons into nonNoci (Fig. 1E) and Noci neurons (Fig. 1F). For MI neurons, most (88%, n = 14) of them were Noci neurons and the remaining 12% (n = 2) were nonNoci neurons (Fig. 1G). For MS neurons, 78% (n = 57) of them were Noci neurons and 22% (n = 16) were nonNoci neurons (Fig. 1H).

MA currents recorded from our acutely dissociated DRG neurons showed a broad range of kinetics from rapidly adapting (RA, Fig. 2A), intermediate adapting (IA, Fig. 2B), to slowly adapting (SA, Fig. 2C). The histogram (n = 73) of MA current decay time constants showed a distribution skewed to the cells with the RA current type (Fig. 2D). There was no discretionary subpopulations of MS cells that could be identified from the time constant histogram (Fig. 2D). We arbitrarily classified MA currents with $\tau < 10$ ms as RA current, $\tau = 10 - 50$ ms as IA currents, $\tau > 50$ ms as SA currents. Using this classification, most (59%, n = 43) of our MS cells showed RA currents; 30% and 8% of our MS cells displayed IA and SA currents, respectively (Fig. 2E). The averaged decay time constants (Fig. 2F) for each of the RA, IA, and SA cell categories were 5.85 ± 0.39 ms (n = 43), 17.51 ± 1.6 ms (n = 22), and 96.79 ± 15.14 ms (n = 8), respectively. When these MS cells were sub-classified into nonNoci and Noci neurons, it was found that