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Research report

Acid modulation of tetrodotoxin-sensitive Na^+ channels in large-sized trigeminal ganglion neurons

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ABSTRACT

Voltage-gated Na^+ channels in primary afferent neurons can be divided into tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) Na^+ channels. Although previous studies have shown the acid modulation of TTX-R Na^+ channels, the effect of acidic pH on tetrodotoxin-sensitive (TTX-S) Na^+ channels is still unknown. Here we report the effect of acidic pH on TTX-S Na^+ channels expressed in large-sized trigeminal ganglion (TG) neurons using a whole-cell patch clamp technique. The application of acidic extracellular solution decreased the peak amplitude of TTX-S currents (I_{Na}) in a pH-dependent manner, but weak acid ($\geq$ pH 6.0) had no inhibitory effect on TTX-S I_{Na}. Acidic pH (pH 6.0) shifted both the activation and steady-state fast inactivation relationships of TTX-S Na^+ channels toward depolarized potentials. However, acidic pH (pH 6.0) had no effect on use-dependent inhibition in response to high-frequency stimuli, development of inactivation, and accelerated the recovery from inactivation of TTX-S Na^+ channels, suggesting that TTX-S Na^+ channels in large-sized TG neurons are less sensitive to acidic pH. Given that voltage-gated Na^+ channels play a pivotal role in the generation and conduction of action potentials in neural tissues, the insensitivity of TTX-S Na^+ channels expressed in large-sized TG neurons to acidic pH would ensure transmission of innocuous tactile sensation from orofacial regions at acidic pH conditions.

1. Introduction

Primary afferent neurons in the dorsal root ganglia (DRG) and trigeminal ganglia (TG) can be largely classified into three types based on their morphological, immunohistochemical and electrophysiological properties; C-, Aδ- or Aβ-type afferents (Mense, 1990; Lawson, 2002; Le Pichon and Chesler, 2014). C-type neurons transmit polymodal nociceptive (thermal and mechanical) signals and Aδ-type neurons mainly transmit mechanical pain mediated by high-threshold mechanoreceptors (Christensen and Corey, 2007; Delmas et al., 2011), and together these two types of neurons transmit nociceptive signals. While a subset of both Aδ-type and C-type neurons is known to transmit tactile sensation, Aβ-type neurons mainly decode various tactile sensation mediated by low-threshold mechanoreceptors in response to innocuous stimuli (Abraira and Ginty, 2013).

Sensory information generated from peripheral terminals of sensory neurons is conducted to the central nervous system by action potentials through afferent nerve fibers. Therefore, voltage-gated ion channels, especially voltage-gated Na^+ channels, play a crucial role in the transmission of sensory information. Sensory neurons differentially express at least six types of voltage-gated Na^+ channels, which can be divided into tetrodotoxin-sensitive (TTX-S: $Na_V1.1$, $Na_V1.2$, $Na_V1.6$, and $Na_V1.7$) and tetrodotoxin-resistant (TTX-R: $Na_V1.8$ and $Na_V1.9$) subtypes (Amaya et al., 2000; Caffrey et al., 1992; Chahine and O'Leary, 2014; Dib-Hajj et al., 1998; Elliott and Elliott, 1993; Ho and O'Leary, 2011; Roy and Narahashi, 1992). It is generally accepted that large-sized sensory neurons express TTX-S Na^+ channels, and that small- and middle-sized sensory neurons express both TTX-S and TTX-R Na^+ channels, although several previous studies have shown the existence of TTX-R Na^+ channels in large-sized sensory neurons (Amaya et al., 2000; Dib-Hajj et al., 1998; Novakovic et al., 1998; Renganathan et al., 2000; Sangameswaran et al., 1996).

Extracellular pH is tightly regulated within a narrow range, but it can fall down as low as 5.4 under several pathological

Abbreviations: ASICs, acid-sensing ion channels; DRG, dorsal root ganglia; I_{Na}, Na^+ currents; $[Na^+]_o$, extracellular Na^+ concentration; τ_{fast}, fast time constants; τ_{slow}, slow time constants; τ_{WD} or $\tau_{weighted}$, weighted time constant; TG, trigeminal ganglia; TTX-R, tetrodotoxin-resistant; TTX-S, tetrodotoxin-sensitive; V_H, holding potential

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conditions, such as inflammation and ischemia (Reeh and Steen, 1996). The resultant local tissue acidosis may change the excitability of primary afferent neurons, but the role of tissue acidosis in nociceptive transmission has been emphasized (Steen and Reeh, 1993; Steen et al., 1992; 1995a; 1995b). This is because acidic pH directly activates acid-sensing ion channels as well as transient receptor potential vallinoid 1, and can modulate a number of ion channels, which are involved in nociceptive transmission (Wemmie et al., 2006; Holzer, 2009). In this regard, we have recently shown that acidic pH modulates several properties of TTX-R Na^+ channels in small-sized TG neurons (Nakamura and Jang, 2015). However, the effect of acidic pH on TTX-S Na^+ channels in TG neurons should be also determined, as TTX-S Na^+ channels are expressed in all sensory neurons regardless of their size and changes in the properties of these channels in TG neurons may affect the perception of sensory information in response to various external stimuli from orofacial region. In the present study, therefore, we examined whether acidic pH changes the basic properties of TTX-S Na^+ channels in large-sized TG neurons, because it is experimentally difficult to separate TTX-S Na^+ currents in small-sized TG neurons.

2. Results

2.1. Effects of acidic pH on TTX-S Na^+ channels

The TTX-S I_{Na} was recorded by depolarizing step pulses (–100 mV to −20 mV every 5 s) in the presence of 100 μM Cd^{2+} from large-sized [> 40 μm in a diameter (106.1 ± 18.5 pF, n = 58, standard deviation)] TG neurons (Fig. 1A, B). The application of 300 nM TTX completely abolished the I_{Na} in large-sized TG neurons (Fig. 1Bb), indicating that TTX-R Na^+ channels are not expressed in these large-sized TG neurons. In contrast, the TTX-R I_{Na} was recorded following depolarizing step pulses (–80 mV to −10 mV every 5 s) in the presence of both 300 nM TTX and 100 μM Cd^{2+} from small-sized [< 30 μm in a diameter (23.5 ± 5.9 pF, n = 42, standard deviation)] TG neurons (Fig. 1A, B). The activation rate of TTX-S I_{Na}, which was measured as the time to peak of I_{Na}, was much faster than that of TTX-R I_{Na} (0.22 ± 0.02 ms for TTX-S I_{Na} and 0.75 ± 0.06 ms for TTX-R I_{Na}, n = 9, p < 0.05, ANOVA, Fig. 1C). The inactivation rate from open state of TTX-S I_{Na}, which was measured as the weighted decay time constant (τ_{WD}), was much faster than that of TTX-R I_{Na} (1.1 ± 0.1 ms for TTX-S I_{Na} and 2.6 ± 0.2 ms for TTX-R I_{Na}, n = 9, p < 0.05, ANOVA, Fig. 1C). The application of acidic extracellular solution decreased the peak amplitude of TTX-S I_{Na} in a pH-dependent manner, where pH 6.0 solution decreased the peak amplitude of TTX-S I_{Na} to 91.8 ± 2.2% of the control (n = 9, p < 0.01) (Fig. 1D, E). The half-inhibitory pH (IC_{50}) for TTX-S Na^+ channels was 5.0 ± 0.1 (n = 7), and this value was significantly lower than that for TTX-R Na^+ channels (IC_{50}: 5.2 ± 0.1, n = 7, p < 0.05, ANOVA) (Fig. 1E). The acidic pH did not affect the τ_{WD} of TTX-S I_{Na} (1.1 ± 0.1 ms for the control and 1.2 ± 0.1 ms for pH 6.0 solution, n = 9, p = 0.74, Fig. 1F). In following all electrophysiological recordings from large-sized neurons, we confirmed whether 300 nM TTX substantially blocked the TTX-S I_{Na} at the end of individual experiments.

2.2. Effects of acidic pH on the voltage-dependence of TTX-S Na^+ channels

The voltage-activation relationship of voltage-dependent Na^+ channels was examined from large- and small-sized TG neurons. In these experiments, the extracellular Na^+ concentration was decreased from 120 mM to 30 mM. TTX-S or TTX-R Na^+ channels were activated by 50 ms depolarizing test pulses from V_Hs (–100 mV or −80 mV for TTX-S or TTX-R Na^+ channels, respectively) in 10 mV increments (Fig. 2A). Fig. 2B shows the current-voltage relationships and conductance-voltage relationships of

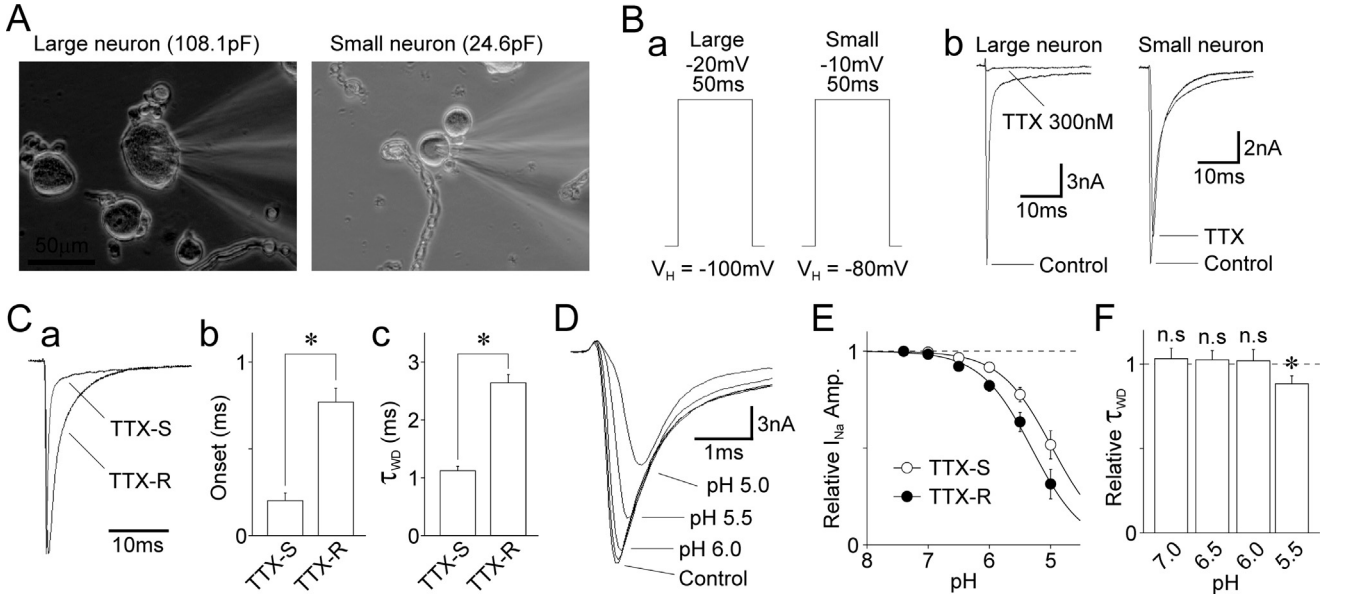

Fig. 1. Effect of acidic pH on TTX-S I_{Na}. A, Phase contrast images of large-sized (left) or small-sized (right) TG neurons. The membrane capacitance was determined using pClamp software during the whole-cell patch clamp recording. Ba, Schematic illustrations of voltage step pulses to elicit the I_{Na} in large-sized (left) or small-sized (right) TG neurons. In large-sized TG neurons, the TTX-S I_{Na} was elicited using electrical stimulation from a V_H of −100 mV to −20 mV (50 ms duration) in every 5 s (0.2 Hz). In small-sized TG neurons, the TTX-R I_{Na} was elicited using electrical stimulation from a V_H of −80 mV to −10 mV (50 ms duration) in every 5 s (0.2 Hz). b, Typical traces of TTX-S or TTX-R I_{Na} induced by step pulses in the absence and presence of 300 nM TTX. Note that TTX completely blocked the I_{Na} in small-sized TG neurons. C, Typical traces of TTX-S and TTX-R I_{Na} induced by single step pulses (a). Changes in the onset time (time to peak, a) and the weighted decay time constant (τ_{WD}, b) of TTX-S or TTX-R I_{Na}. *; p < 0.05. D, Typical traces of TTX-S I_{Na} induced by step pulses at various extracellular pH. E, Acidic pH-inhibition relationship of TTX-S or TTX-R Na^+ channels. The continuous curves for acidic pH-inhibition relationship were fitted using a least-squares method. Each point represents the mean and SEM from 8 to 12 experiments. F, Acidic pH-induced changes in the τ_{WD} of TTX-S I_{Na}. Each column represents the mean and SEM from 8 to 12 experiments.*, p < 0.05; n.s, not significant.