Neuropharmacology 90 (2015) 82–89

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Acid modulation of tetrodotoxin-resistant Na^+ channels in rat nociceptive neurons

Michiko Nakamura [a], Il-Sung Jang [a, b, *]

[a] Department of Pharmacology, School of Dentistry, Kyungpook National University, Daegu 700-412, Republic of Korea
[b] Brain Science & Engineering Institute, Kyungpook National University, Daegu 700-412, Republic of Korea

ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form
6 October 2014
Accepted 13 November 2014
Available online 28 November 2014

Keywords:
Acidosis
Inflammatory pain
TTX-R Na^+ channels
Trigeminal ganglia
Patch clamp

ABSTRACT

Under pathological conditions including inflammation, ischemia and incision, extracellular pH falls down as low as 5.4. Although some mediators play pivotal roles in the development and maintenance of inflammatory hyperalgesia by affecting the functional properties of tetrodotoxin-resistant (TTX-R) Na^+ channels, the roles of tissue acidosis in nociceptive transmission mediated by TTX-R Na^+ channels are largely unknown. In the present study, we have investigated the effect of acidic pH on TTX-R Na^+ currents (I_{Na}) in small-sized sensory neurons isolated from rat trigeminal ganglia using a whole-cell patch clamp technique. Acidic pH decreased the peak amplitude of TTX-R I_{Na} in a pH-dependent manner, but weak acid (≥pH 6.0) had a minor inhibitory effect on the TTX-R I_{Na}. Acidic pH also significantly shifted both the activation and steady-state fast inactivation relationships toward depolarized potentials. In addition, acidic pH had little effect on the use-dependent inhibition, and significantly retarded the development of inactivation and accelerated the recovery from inactivation of TTX-R Na^+ channels. The results suggest that weak acid (≥pH 6.0) makes TTX-R Na^+ channels to be suitable for the repetitive activation at depolarized membrane potentials. Given that both tissue acidosis and inflammatory mediators in inflamed or injured tissues act synergistically to promote nociceptive transmission by affecting the functional properties of TTX-R Na^+ channels, these channels would be, at least in part, a good target to treat inflammatory pain.

1. Introduction

In the nervous system, extracellular pH is tightly regulated to maintain the normal neuronal function. Under pathological conditions including inflammation, ischemia, incision and malignant tumors, however, extracellular pH falls down as low as 5.4 (for review, Reeh and Steen, 1996). Extracellular pH also falls down even in the normal physiological conditions such as spontaneous neuronal activity (Chesler and Kaila, 1992). The resultant tissue acidosis might have a broad impact on electrically excitable tissues including nerve, heart and muscle (de Hemptinne et al., 1987; Chesler and Kaila, 1992). For example, a decrease in extracellular pH around peripheral tissues can produce pain by activating nociceptive neurons, as the injection of acidic pH solution elicits not only mechanical and thermal hyperalgesia but non-desensitizing nociceptor activation (Steen and Reeh, 1993; Steen et al., 1992, 1995a). Therefore, local tissue acidosis may contribute to the development of painful states such as inflammation and incision (Steen et al., 1995b; Woo et al., 2004). In peripheral tissues, acidic pH can directly excite nociceptive neurons by activating acid-sensing ion channels (ASICs), which are Na^+-selective cation channels, and therefore ASICs are considered as one of key ion channels to excite nociceptive neurons (Krishtal, 2003; Wemmie et al., 2006, 2013). However, since a number of ion channels and receptors, such as voltage-gated Na^+, K^+ and Ca^{2+} channels and transient receptor potential vanilloid 1 (TRPV1), can be also regulated by acidic pH (Wemmie et al., 2006; Holzer, 2009), the roles of acidic pH in nociceptive transmission would be rather complicated.

Tetrodotoxin-resistant (TTX-R) Na^+ channels, e.g., $Na_V1.8$ and $Na_V1.9$, are expressed on small- and medium-sized sensory neurons within the dorsal root and trigeminal ganglia, and have been

Abbreviations: ASICs, acid-sensing ion channels; 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.

* Corresponding author. Department of Pharmacology, School of Dentistry, Kyungpook National University, 2177 Dalgubeol-daero, Jung-gu, Daegu 700-412, Republic of Korea. Tel.: +82 53 660 6887; fax: +82 53 424 5130.
E-mail address: jis7619@knu.ac.kr (I.-S. Jang).

http://dx.doi.org/10.1016/j.neuropharm.2014.11.005
0028-3908/

implicated in inflammatory and neuropathic pain as well as cold pain (Zimmermann et al., 2007; Eijkelkamp et al., 2012; Waxman and Zamponi, 2014). TTX-R Na^+ channels are activated and inactivated at more depolarized potentials compared to TTX-sensitive (TTX-S) Na^+ channels (Elliott and Elliott, 1993). These properties endow TTX-R Na^+ channels with ideality to generate and conduct action potentials in damaged sensory neurons, where the resting membrane potential is somewhat persistently depolarized (Schild and Kunze, 1997). In fact, the slowly inactivating TTX-R Na^+ currents significantly contribute to the generation of action potentials (Renganathan et al., 2001), suggesting that TTX-R Na^+ channels may play pivotal roles in nociceptive transmission. On the other hand, a few studies have shown the effect of acidic pH on voltage-gated Na^+ channels. In hippocampal neurons, for example, moderate acidosis depresses TTX-S Na^+ currents by shifting voltage dependence of activation but not steady-state inactivation (Tombaugh and Somjen, 1996). A recent study has also shown that acidosis differentially modulates the biophysical properties of some Na^+ channel subtypes (Vilin et al., 2012). However, it is poorly known whether acidic pH directly modulates TTX-R Na^+ channels. In the present study, therefore, we have investigated the effect of acidic pH on the currents mediated by TTX-R Na^+ channels in rat trigeminal sensory neurons.

2. Materials and methods

2.1. Preparation

All experiments complied with the guiding principles for the care and use of animals approved by Kyungpook National University and the Council of the Physiological Society of Korea, and every effort was made to minimize both the number of animals used and their suffering.

Sprague Dawley rats (5–6 week old, either sex) were decapitated under ketamine anesthesia (100 mg/kg, ip). The trigeminal ganglia (TG) were dissected and treated with an external solution (in mM; 150 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 Hepes, a pH of 7.4 with Tris-base) containing 0.3% collagenase (type I) and 0.3% trypsin (type I) for 30–40 min at 37 °C. Thereafter, TG neurons were dissociated mechanically by triturating with fire-polished Pasteur pipettes in a culture dish (Primaria 3801, Becton Dickinson, Rutherford, NJ, USA). The isolated TG neurons were used for electrophysiological experiments 1–6 h after preparation.

2.2. Electrical measurements

All electrical measurements were performed using conventional whole-cell patch recordings and a patch-clamp amplifier (Axopatch 200B; Molecular Devices, Union City, CA, USA) with a K^+-free external solution [in mM; 130 NaCl, 20 TEA-Cl, 3 CsCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose and 10 HEPES (pH 6.0–7.4) or 10 MES (pH 5.5–5.0) and was adjusted to a pH with Tris-base]. In a subset of experiments, 100 mM NaCl was replaced with equimolar N-methyl-glucamine-Cl. Neurons were voltage clamped at a holding potential (V_H) of −80 mV, except where indicated. Patch pipettes were made from borosilicate capillary glass (G-1.5; Narishige, Tokyo, Japan) by use of a pipette puller (P-97; Sutter Instrument Co., Novato, CA, USA). The resistance of the recording pipettes filled with the internal solution (in mM; 135 CsF, 10 CsCl, 2 EGTA, 4 ATP-Na_2 and 10 Hepes with a pH adjusted to 7.2 with Tris-base, except where indicated) was 0.8–1.5 MΩ. Membrane potentials were corrected for the liquid junction potential (~−11 mV, measured by exchanging bath solution from internal solution to standard external solution) and the pipette capacitance and series resistance (40–80%) were compensated for. Neurons were viewed under phase contrast on an inverted microscope (TE-2000; Nikon, Tokyo, Japan). Membrane currents were filtered at 5 kHz, digitized at 20 kHz, and stored on a computer equipped with pCLAMP 10.3 (Molecular Devices). Capacitative and leakage currents were subtracted by the P/4 protocol using pCLAMP program. During recordings, 10 mV hyperpolarizing step pulses (30 ms in duration) were periodically applied to monitor the access resistance, and recordings were discontinued if access resistance changed by more than 10%. All experiments were performed at room temperature (22–25 °C). To record TTX-R Na^+ currents (I_{Na}), the K^+-free external solution routinely contained 500 nM TTX and 200 μM Cd^{2+} to block TTX-sensitive (TTX-S) Na^+ channels and voltage-gated Ca^{2+} channels. The all extracellular solutions were applied using the 'Y–tube system' for rapid solution exchange (Murase et al., 1989). All drugs used in this study were purchased from Sigma (St. Louis, MO, USA).

2.3. Data analysis

The amplitude of TTX-R Na^+ currents (I_{Na}) was measured by subtracting the baseline from the peak amplitude of TTX-R I_{Na} by using Clampfit program (Molecular Devices). The continuous curves for the concentration–response relationship were fitted using a least-squares fit to the following equation, $I = 1 - [C^n/(C^n + EC_{50}^n)]$, where I is the acidic pH-induced inhibition of TTX-R I_{Na}, C is the proton concentration, EC_{50} is the pH for the half-effective response and n is the Hill coefficient. The amplitude of TTX-R I_{Na}, was transformed into conductance (G) using the following equation; $G = I/(V - E_{Na})$, where E_{Na} is the Na^+ equilibrium potential (+33.3 mV) calculated by the Nernst equation. The voltage-activation and voltage-inactivation relationships of TTX-R Na^+ channels were fitted to the Boltzmann equations, respectively; $G/G_{max} = 1/\{1 + \exp[(V_{50,act} - V)/k]\}$ and $I/I_{max} = 1-1/\{1 + \exp[(V_{50,inact} - V)/k]\}$, where G_{max} and I_{max} are the maximum conductance and current amplitude, $V_{50,act}$ and $V_{50,inact}$ are half-maximum potentials for activation and fast inactivation, and k is the slope factor. The fast (τ_{fast}) and slow time constants (τ_{slow}) of the decay of single TTX-R I_{Na} and the kinetic data for the recovery from inactivation were fitted to the following equation; $I(t) = A_0 + A_{fast} \times [1 - \exp(-t/\tau_{fast})] + A_{slow} \times [1 - \exp(-t/\tau_{slow})]$, and the kinetic data for the development of inactivation were fitted to the following equation; $I(t) = A_0 + A_{fast} \times [\exp(-t/\tau_{fast})] + A_{slow} \times [\exp(-t/\tau_{slow})]$, where $I(t)$ is the amplitude of TTX-R I_{Na} at time t, and A_{fast} and A_{slow} are the amplitude fraction of τ_{fast} and τ_{slow}, respectively. The weighted time constant (τ_{WD} or $\tau_{weighted}$) was calculated by using the following equation; τ_{WD} or $\tau_{weighted} = [(\tau_{fast} \times A_{fast}) + (\tau_{slow} \times A_{slow})]/(A_{fast} + A_{slow})$. Numerical values are provided as the mean ± SEM using values normalized to the control. Significant differences in the mean amplitude were tested using Student's paired two-tailed t-test, using absolute values rather than normalized ones. Values of $p < 0.05$ were considered significantly different.

3. Results

3.1. Effects of acidic pH on TTX-R Na^+ channels

Previous studies have shown that acidic pH inhibits the current amplitude mediated by voltage-gated Na^+ channels in several preparations (Hille, 1968; Tombaugh and Somjen, 1996; Daumas and Andersen, 1993; Khan et al., 2002). Therefore, we firstly examined the effects of acidic pH on the peak amplitude of TTX-R I_{Na}. The TTX-R I_{Na} was recorded by depolarizing step pulses (−80 to −10 mV at every 5 s) in the presence of 500 nM TTX and 200 μM Cd^{2+} from small-sized [(20–25 μm in a diameter (25.9 ± 6.1 pF; standard deviation from 173 neurons)] TG neurons. The application of pH 6.0 solution decreased the peak amplitude of TTX-R I_{Na} to 83.4 ± 5.7% of the control ($n = 8$, $p < 0.01$, Fig. 1A, B). The pH 6.0 solution-induced decrease in TTX-R I_{Na} was not affected by the concentration of TTX (30 nM–1 μM) or Cd^{2+} (30–300 μM) (data not shown). The acidic pH reduced the peak amplitude of TTX-R I_{Na} in a pH-dependent manner with a half-inhibitory pH (IC_{50}) of 5.28 ± 0.12 ($n = 8$, Fig. 1B). While the activation rate of TTX-R I_{Na}, which was measured as the time to peak of Na^+ currents, was delayed by acidic pH (0.66 ± 0.03 ms for the control and 0.94 ± 0.04 ms for pH 6.0 solution, 141.5 ± 3.1% increase, $n = 8$, $p < 0.01$, Fig. 1Ca), the inactivation rate from open state of TTX-R I_{Na}, which was measured as the weighted decay time constant (τ_{WD}), was not affected by weak acidic pH (≥pH 6.0) (2.42 ± 0.12 ms for the control and 2.55 ± 0.10 ms for pH 6.0 solution, 106.1 ± 2.8% change, $n = 8$, $p < 0.01$, Fig. 1Cb). However, the application of pH 5.5 solution greatly increased the τ_{WD} to 215.9 ± 25.3% of the control ($n = 8$, $p < 0.01$, Fig. 1Cb).

On the other hand, the application of acidic pH solution elicited the fast and rapidly desensitizing inward currents in a subset of TG neurons (36 of 188 TG neurons tested, 19.1%, Fig. 1Da). These inward currents were substantially blocked by 300 μM amiloride, a nonselective ASIC inhibitor (Fig. 1Da). However, since the ASIC currents were rapidly desensitized within 5 s from the application, and the membrane properties including the input resistance were not affected during the prolonged application of pH 5.5 solution (231.5 ± 24.9 MΩ for the control and 215.9 ± 35.1 MΩ during pH 5.5 solution, $n = 6$, $p = 0.84$), there was no difference in the extent of acid modulation of TTX-R Na^+ channels regardless of the existence of amiloride-sensitive ASIC currents. In following all experiments, therefore, the acid effect on TTX-R Na^+ channels was examined 20 s after the application of acidic pH solution. Another important acid-sensitive ion channel is TRPV1 (Holzer, 2009), and therefore acidic