Neuroscience Letters 559 (2014) 30–33

Contents lists available at ScienceDirect

Neuroscience Letters

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

Persistent sodium current properties in hippocampal CA1 pyramidal neurons of young and adult rats

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HIGHLIGHTS

- Persistent sodium current is an important contributor to cellular and network excitability.
- We analyzed changes in I_{NaP} properties during maturation.
- CA1 pyramidal cells were isolated from hippocampus of P12-16 and P60-75 rats.
- I_{NaP} density is substantially increased in the adult group.
- Hyperpolarization shift of I_{NaP} activation with increasing postnatal age.

ARTICLE INFO

Article history:
Received 12 July 2013
Received in revised form
14 November 2013
Accepted 18 November 2013

Keywords:
Persistent tetrodotoxin-sensitive sodium current
Hippocampal CA1 pyramidal neurons
Postnatal development

ABSTRACT

Persistent tetrodotoxin-sensitive sodium current (I_{NaP}) plays an important role in cellular and neuronal network excitability in physiological conditions and under different pathological circumstances. However, developmental changes in I_{NaP} properties remain largely unclear. In the present study using whole cell patch clamp technique we evaluated I_{NaP} properties in CA1 hippocampal pyramidal neurons isolated from young (postnatal day (P) 12–16) and adult (P60-75) rats. We show that the I_{NaP} density is substantially larger in the adult group. Although I_{NaP} inactivation characteristics were found to be similar in both groups, voltage dependence of I_{NaP} activation is shifted to more negative membrane potentials (young: -48.6 ± 0.5 mV vs. adult: -52.4 ± 0.2 mV, $p < 0.01$). Our data indicates the increase of I_{NaP} contribution in the basal membrane sodium conductivity in the mature hippocampus.

1. Introduction

Slowly inactivating "persistent" Na^+ current (I_{NaP}) mediated by sodium channels with activation near the resting membrane potential was found in various central and peripheral neurons including neocortex, entorhinal cortex, thalamus, cerebellum, hippocampus and dorsal root ganglia (see [1,2] for ref). I_{NaP} plays an important role in subthreshold oscillations of membrane potential and sustains recurrent firing in response to membrane depolarization [3–5]. Moreover, I_{NaP} can amplify synaptic potentials and contributes to intrinsic pacemaking activity of different neurons [6–8]. Several reports indicate that pathological conditions, such as chronic epilepsy and hypoxia, can be accompanied by enhancement of I_{NaP} conductance implying involvement of this current in pathological synchronization [9–11]. Despite the significant role of I_{NaP} in the neuronal function little is known about age dependent changes in its properties. During last three decades after discovering of I_{NaP} only few studies were devoted to investigation of developmental changes in the expression of this current. Alzheimer with colleagues [12] showed threefold increase in the I_{NaP} density during first three postnatal weeks in pyramidal neurons of sensorimotor cortex. Authors suggested that I_{NaP} plays an important role in the control of excitability during early postnatal development. This data is in agreement with our previous report where activation of I_{NaP} substantially facilitated seizure-like activity in immature hippocampus [13]. However, developmental changes in I_{NaP} properties through the late adolescent period remain unclear. In the present study, patch clamp recordings were made from hippocampal CA1 pyramidal cells isolated from rats at the second postnatal week (postnatal days (P) 12–16) and at P60-75. Here we demonstrate substantial changes in both current density and steady-state activation of I_{NaP} during maturation.

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http://dx.doi.org/10.1016/j.neulet.2013.11.035

2. Materials and methods

All experimental procedures were performed in accordance with the guidelines set by the National Institutes of Health for the humane treatment of animals and approved by the Animal Care Committee of Bogomoletz Institute of Physiology.

2.1. Slices and acutely isolated neurons preparations

The hippocampal slice preparation and neuron dissociation procedure was performed as described previously with some modifications [14]. Briefly, young (P12-16, $N = 19$) and adult (P60-75, $N = 23$) Wistar rats were anesthetized with sevoflurane, decapitated and brain was rapidly moved into ice-cold oxygenated (95% O_2, 5% CO_2) dissecting solution contained (in mM): 130 NaCl, 5 KCl, 0.1 $CaCl_2$, 5 $MgCl_2$, 1 NaH_2PO_4, 1 Na_2HPO_4, 26 $NaHCO_3$, 10 glucose. Hippocampal slices (400–500 μm) were cut using a vibratome (MA752, Campden Instrument, Loughborough, UK) and kept for at least an hour at room temperature (22–24 °C) in incubation solution contained (in mM): 130 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 1 NaH_2PO_4, 1 Na_2HPO_4, 26 $NaHCO_3$, 10 glucose. Slices were then transferred to sucrose-based solution contained (in mM): 290 sucrose, 3 KCl, 0.5 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 15 glucose, 2 mg/ml pronase E (32 °C). After 15 min of enzymatic treatment slices were rinsed with artificial cerebrospinal fluid (ACSF) contained (in mM): 140 NaCl, 5 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 20 HEPES (pH adjusted to 7.4 by NaOH). Isolation of neurons from pyramidal CA1 region of hippocampus was performed using vibrodissociation technique [14].

2.2. Electrophysiological recordings

Conventional patch clamp technique in the whole cell configuration was used for the recording of I_{NaP} in acutely isolated pyramidal neurons. Extracellular solution consisted (in mM): 130 NaCl, 2 $CaCl_2$, 1.3 $MgCl_2$, 20 TEACl, 10 HEPES and 0.4 $CdCl_2$ (pH was adjusted to 7.35 with NaOH). Patch pipettes were filled with an intracellular solution contained (in mM): 120 CsF, 5 NaCl, 30 TEA-Cl, 10 EGTA, 10 Tris-HCl (pH was adjusted to 7.2 with CsOH). Pipette resistances were ranged from 3 to 5 MΩ. All recordings were made using model 2400 patch clamp amplifier (A-M Systems, Carlsborg, WA). Recordings were digitized at 2 kHz by analogue-to-digital converter (NI PCI-6221, National instruments, Austin, TX, USA) and filtered offline for further analysis. Liquid junction potential was determined and command voltages were corrected accordingly. All experiments were made at the room temperature. I_{NaP} was elicited by slow depolarizing ramp voltage protocol (from −80 mV to 0 mV, 30 mV/s). Current–voltage relationships of I_{NaP} were obtained by subtraction of five averaged recordings before and after treatment with 1 μM tetrodotoxin (TTX). The capacitance of cells was estimated from the change in membrane charge, determined from the integrated capacity transients evoked by a 10 mV hyperpolarizing step from a holding potential (V_h) of −80 mV. Voltage dependence of I_{NaP} conductance (G) was calculated from $I/(V - V_{rev})$, where I is the amplitude of I_{NaP} during the test depolarization (V), and V_{rev} is the Na^+ reversal potential calculated from the Nernst equation for the given extra- and intracellular Na^+ concentrations (82 mV throughout). To evaluate the voltage-dependence of I_{NaP} steady-state inactivation the conditioning prepulses varied from −80 to −10 mV (10 s duration, 10 mV step) were preceded each standard voltage ramp stimulation to elicit I_{NaP}. All voltage-dependence relationships were normalized to the maximal conductance for each recording, then summarized for each group and fitted with a Boltzmann function. The time-dependence of inactivation was evaluated by applying conditioning prepulses to −10 mV at various duration (from 100 ms to 10 s) before a voltage ramp protocol eliciting I_{NaP}. Obtained data were summarized and fitted with mono exponential function.

2.3. Data analysis

Statistical data analysis was performed using Prism 5 (GraphPad, La Jolla, CA) and Origin 7.5 (OriginLab, Northampton, MA) software. The Shapiro–Wilk test was used to estimate normality of the data for each group. Statistical analysis of the development changes in I_{NaP} amplitude and density was performed using unpaired two-sample Student's t-test. Extra sum-of-squares F-test was used for voltage dependence of I_{NaP} conductance fit comparison. All data in the text were presented as the mean ± SE.

2.4. Chemicals

TTX was obtained from Tocris (Ellisville, MO). All other chemicals were purchased from Sigma (St. Louis, MO).

3. Results

Whole cell I_{NaP} were elicited in isolated hippocampal CA1 pyramidal cells of young (P12-16) and adult (60–75) rats using slow ramp depolarization from holding potential −80 mV to 0 mV (30 mV/s) [13]. Fig. 1A shows five superimposed responses evoked

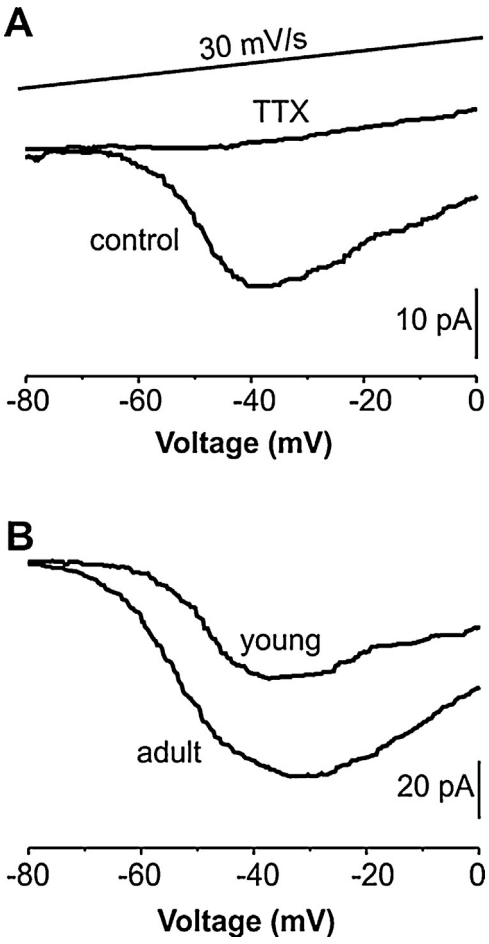

Fig. 1. Persistent tetrodotoxin-sensitive Na^+ current in hippocampal CA1 pyramidal neurons. (A) Representative averaged currents of five superimposed traces evoked by ramp voltage depolarization from −80 mV to 0 mV before and after exposure to 1 μM TTX. (B) Examples of I_{NaP} measured in hippocampal CA1 pyramidal neurons isolated from P12 (1) and P67 (2) rats (current traces were obtained by subtraction of TTX resistant current from total current).