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Research Report

Extracellular sodium modulates the excitability of cultured hippocampal pyramidal cells

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ABSTRACT

Recent studies demonstrated a photophobia mechanism with modulation of nociceptive, cortico-thalamic neurons by retinal ganglion cell projections; however, little is known about how their neuronal homeostasis is disrupted. Since we have found that lumbar cerebrospinal fluid (CSF) sodium increases during migraine and that cranial sodium increases in a rat migraine model, the purpose of this study was to examine the effects of extracellular sodium ($[Na^+]_o$) on the intrinsic excitability of hippocampal pyramidal neurons. We monitored excitability by whole cell patch using a multiplex micropipette with a common outlet to change artificial CSF (ACSF) $[Na^+]_o$ at cultured neurons accurately (SD<7 mM) and rapidly (<5 s) as determined by a sodium-selective micro-electrode of the same size and at the same location as a neuronal soma. Changing $[Na^+]_o$ in ACSF from 100 to 160 mM, choline-balanced at 310–320 mOsm, increased the action potential (AP) amplitude, decreased AP width, and augmented firing rate by 28%. These effects were reversed on returning the ACSF $[Na^+]_o$ to 100 mM. Testing up to 180 mM $[Na^+]_o$ required ACSF with higher osmolarity (345–355 mOsm), at which the firing rate increased by 36% between 100 and 180 mM $[Na^+]_o$, with higher amplitude and narrower APs. In voltage clamp mode, the sodium and potassium currents increased significantly at higher $[Na^+]_o$. These results demonstrate that fluctuations in $[Na^+]_o$ modulate neuronal excitability by a sodium current mechanism and that excessively altered neuronal excitability may contribute to hypersensitivity symptoms.

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1. Introduction

Fluctuating excitability is a fundamental characteristic of neurons, and its homeostatic regulation depends on the intrinsic properties of the neuronal membrane, its many synaptic inputs, and the local environment. Failed homeostasis may result in symptoms from excessive excitability, such as epilepsy and the sensory disturbances of migraine, or from

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Abbreviations: ACSF, artificial CSF; AHP, after hyperpolarization; AP, action potential; CSF, cerebrospinal fluid; DIV, days in vitro; E_{Na}, sodium reversal potential; h current, hyperpolarization-activated cation current; Kdr, delayed rectifier potassium current; $[Na^+]_o$, extracellular sodium concentration; RMP, resting membrane potential; SD, standard deviation; SSME, sodium-selective micro-electrode; TBST, Tris-buffered saline with 0.05% Tween-20; TTX, tetrodotoxin

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decreased excitability, such as depression. For example, the recently reported neuronal pathway for migraine photophobia (Noseda et al., 2010) must be more excitable to relay the discomfort induced by normal ambient light, though the basis for this excess excitability is not known. Regaining neuronal excitability homeostasis is central to the treatment of these disorders and this may only be achieved with a more complete understanding of how homeostasis fails.

A study of relevant cations that may affect neuronal excitability in the failed homeostasis of migraine highlighted sodium deviation (Harrington et al., 2006b) of lumbar cerebrospinal fluid (CSF) levels of potassium, calcium, sodium, and magnesium, only sodium changed (increased) during migraine. This change was confined to the central nervous system (blood plasma levels were unchanged) and CSF and blood osmolarity did not change. We recently performed ^{23}Na MRI in a rat migraine model (Harrington et al., 2011): cranial sodium increased by 7–17% in synchrony with behavioral manifestations of central sensitization and brainstem cFos activation. Furthermore, mathematical simulations revealed that an increase in extracellular sodium, $[Na^+]_o$, equivalent to that observed in the rat model increases the firing frequency of spontaneous action potentials (APs): To build a cell model, we constructed a simple soma from a representative cylinder (diameter: 20 μm; length: 20 μm) and a 50 μs time step. The model cell soma includes sodium current, potassium currents (delayed rectifier, Kdr, and A-type, KA), leak current, Na,K-ATPase, and sodium diffusion. The intracellular resistance, was defined as 150 Ω cm; membrane capacitance as 1 μF cm^{-2}, and the resting membrane potential was defined as −65 mV. At 145 mM $[Na^+]_o$, the model fired at 3.25 Hz and increased by 17% at 165 mM $[Na^+]_o$ to 6.38 Hz (Harrington et al., 2011). This simulation supports the notion that rising $[Na^+]_o$ might increase neuronal excitability.

Sodium homeostasis has long been recognized as essential for neuronal excitability, and sodium permeability increases during the AP (Hodgkin, 1964; Hodgkin and Katz, 1949; Huxley, 1964). Thus, the AP potential peak (where sodium permeability is greater than for other ions) will be close to the sodium reversal potential according to the Nernst equation. This relationship predicts that altered extracellular sodium concentration ($[Na^+]_o$) will change the AP amplitude, a result confirmed experimentally in squid axon (Hodgkin, 1964; Hodgkin and Katz, 1949). Similar results were obtained in myelinated nerve (Huxley and Stampfli, 1951) and skate heart (Seyama and Irisawa, 1967). Furthermore, lower $[Na^+]_o$ decreased the height and rising rate of APs in the smooth muscles of the cat ureter (Kobayashi and Irisawa, 1964) and lowered amplitude and increased the width of APs in rat dorsal root ganglion neurons (Amir et al., 1999). Thus, $[Na^+]_o$ affects AP shape and excitability.

When a neuron is at rest, the Na^+ influx through voltage-gated Na^+ channels is low, as these channels are usually closed or inactivated. However, the channel gate is displaced when $[Na^+]_o$ increases (Kuo and Liao, 2000). Higher $[Na^+]_o$ speeds recovery from the inactivation state, enabling an earlier action potential and leading to hyperexcitability (Kuo and Liao, 2000). Higher sodium induces more sub-threshold oscillations in addition to AP changes, which might play a role in neuropathic pain (Amir et al., 1999).

It would be helpful to extend these observations with experiments to examine more details of the effects of increased $[Na^+]_o$ on the excitability of brain neurons. Hippocampal neuronal excitability is of interest because this region is vulnerable to spreading depression, epilepsy, ischemia, and anoxia (Johnston et al., 1991; Kunkler and Kraig, 2003; Pantoni et al., 2000; Stracciari et al., 2008). The goal of this study was to investigate the effects of $[Na^+]_o$ on the excitability of cultured hippocampal pyramidal neurons.

2. Results

2.1. Primary pyramidal cell culture

The cultured cells were heterogeneous since they were dissociated and cultured from the whole hippocampus. Most are pyramidal cells based on morphology: the cells were phase-bright, with smooth membranes, pyramidal-shaped soma, with one or several thin basal dendrites and one large-diameter apical dendrite, sometimes with branching dendrites (Fig. 1A). Since Na^+, K^+, ATPase is a key regulator of $[Na^+]_o$, we studied Na^+, K^+, ATPase expression in these cultured neurons. The cultured cells expressed Na^+, K^+, ATPase subunits alpha-1 and -3, but not alpha-2, with molecular weight around 112 kDa (arrowed in Fig. 1B) and sodium channels with molecular weight around 230 kDa (data not shown). The sodium channels and Na^+, K^+, ATPase isoforms were visualized by phase shift and specific immunostaining (Fig. 1C; alpha-3 data not shown). Thus, the neurons we studied have pyramidal morphology and express alpha-1 and -3 Na^+, K^+, ATPase and sodium channels.

2.2. Rapid and accurate switching of $[Na^+]_o$ for electrophysiology experiments

Fig. 1E shows that the sodium-selective micro-electrode (SSME) recording of $[Na^+]$ at the neuronal site changed to each intended value within 5 s after each switch. For our series of ACSFs ($n=3$) at normal osmolarity with a source of ACSF $[Na^+]$ of 100, 120, 140, 160 mM, the mean $[Na^+]$ (SD) detected at the soma position was 105.85 (4.02), 119.03 (2.87), 141.75 (0.02), and 156.98 (2.30) mM, respectively. In experiments at higher osmolarity ($n=3$), the source ACSF $[Na^+]$ of 100, 140, 180 mM was detected as a mean of 109.70 (5.03), 141.75 (0.02), and 185.07 (6.54) mM, respectively. Thus, the measured $[Na^+]$ that reached the position of the neuronal soma was close to the $[Na^+]$ in the delivery pipette, and the change was complete and stable within 5 s. The calibration of the SSME from a log scale of the sodium concentration indicated a linear relationship (Fig. 1D).

2.3. Excitability of cultured neurons and $[Na^+]_o$

The hippocampal pyramidal neurons we recorded had a resting membrane potential of -64.52 ± 4.54 mV at 100 mM $[Na^+]_o$, and -63.20 ± 5.37 mV at 160 mM $[Na^+]_o$ ($P>0.05$; $n=7$). Their input resistance was 449.99 ± 312.91 MΩ at a $[Na^+]_o$ of 100 mM and 425.16 ± 304.25 MΩ at a $[Na^+]_o$ of 160 mM ($P>0.05$; $n=7$). These cells did not fire spontaneously; however, a positive current injection induced a train of APs characteristic of hippocampal pyramidal cells (Fig. 2A).

A 3–4 mM increase in $[Na^+]$ in cerebrospinal fluid was reported in migraine (Harrington et al., 2006b) that presumably

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