Effects of Hyposmolar Solutions on Membrane Currents of Hippocampal Interneurons and Mossy Cells In Vitro

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Baraban, Scott C. and Philip A. Schwartzkroin. Effects of hyposmolar solutions on membrane currents of hippocampal interneurons and mossy cells in vitro. J. Neurophysiol. 79: 1108–1112, 1998. Whole cell voltage-clamp recordings in rat hippocampal slices were used to investigate the effect of changes in extracellular osmolarity on voltage-activated potassium currents. Currents were evoked from oriens/alveus (O/A) interneurons, hilar interneurons, and mossy cells. Hyposmolar external solutions produced a significant potentiation of K⁺ currents recorded from O/A and hilar interneurons, but not from mossy cells. Hyposmolar solutions also dramatically potentiated the spontaneous excitatory postsynaptic currents recorded from mossy cells. These results suggest that hippocampal excitability can be modulated by the complex actions exerted by changes in extracellular osmolarity.

Introduction

Osmolarity may play a critical role in regulating excitability in the central nervous system (CNS). Hyposmolar clinical conditions, such as inappropriate antidiuretic hormone secretion and water intoxication, are associated with restlessness and increased seizure susceptibility (Medani 1987). A reduction in extracellular osmolarity in vitro enhances epileptiform activity induced by low calcium (Roper et al. 1992), high potassium (Traynelis and Dingledine 1989), or low magnesium (Andrew et al. 1989). Hyposmotic solutions potentiate stimulation-evoked population responses in the hippocampal slice preparation (Saly and Andrew 1993). Further, Huang et al. (1997) recently showed that lowering extracellular osmolarity enhanced excitatory postsynaptic currents (EPSCs) on CA1 pyramidal neurons.

Given that slice recordings of CA3 and CA1 pyramidal neurons revealed no changes in intrinsic properties during hyposmotic challenges shown to modulate synaptic activity (Ballyk et al. 1991; Saly and Andrew 1993), it is unclear how osmolarity modulates hippocampal excitability. One possibility is that specific subtypes of hippocampal neurons are osmosensitive. Attractive candidates are the nonpyramidal, GABAergic interneurons of hippocampus [e.g., oriens/alveus (O/A) and lacunomus/molecular (L/M) of CA1 and hilar interneurons of the dentate] (for review see Freund and Buzsaki 1996). Inhibitory interneurons exert a significant regulatory control over feed-forward and feedback excitation in the hippocampus (Miles and Wong 1987). Further, the firing activity of a single interneuron can have a dramatic effect on hippocampal excitability because each interneuron synapses onto ~200 pyramidal neurons (Traub and Miles 1991). Hippocampal “mossy” cells are another subtype of nonpyramidal hippocampal neuron that play a critical role in regulating hippocampal excitability (Scharfman and Schwartzkroin 1988). The glutamatergic mossy cells synapse with both interneurons and granule cells in the dentate gyrus (Ribak et al. 1985; Wenzel et al. 1997).

We recently presented evidence to support the hypothesis that a subtype of hippocampal neuron is osmosensitive. Specifically, osmolarity was shown to modulate the neuronal K⁺ channel function of L/M interneurons but not of CA1 or subicular pyramidal neurons (Baraban et al. 1997). To test whether other subtypes of hippocampal interneurons (O/A and hilar) or mossy cells exhibit osmosensitivity, we investigated the effect of hyposmotic challenge on voltage-activated K⁺ currents and spontaneous EPSCs recorded from these neurons.

Methods

Hippocampal slices were prepared from 10- to 17-day-old Sprague-Dawley rat pups, as described previously (Baraban et al. 1997). Normosmolar extracellular solution (300 ± 2 mosM, mean ± SE) consisted of either (in mM) 1) 124 NaCl, 3 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 10 dextrose or 2) 104 NaCl, 3 KCl, 1.25 NaH₂PO₄, 2 MgSO₄, 26 NaHCO₃, 2 CaCl₂, and 10 dextrose with the addition of sucrose. Hyposmolar solutions (265 ± 2 mosM) had the same composition as solution 2 without the addition of sucrose. Results were comparable with the use of osmotic gradients produced by a reduction in either [NaCl] in solution 1 or equi-[NaCl] plus sucrose in solution 2 and were therefore pooled. All solutions were adjusted to pH 7.4, bubbled with 95% O₂-5% CO₂, and perfused at a rate of ~2 ml min⁻¹. Tight-seal whole cell voltage-clamp room temperature recordings were made with an Axopatch 1-D amplifier with appropriate series and capacitance compensation. Patch pipettes containing a potassium gluconate-based internal solution (289 ± 2 mosM) (Baraban et al. 1997) were positioned under visual control using a Zeiss microscope, a water-immersion objective (×40) with Hoffman differential interference contrast optics, and an infrared camera. Potassium currents were leak subtracted. Voltage-clamp command potentials and analysis of currents were performed by using pClamp (Axon Instruments). Current records were low-pass filtered at 2 kHz (~3 dB, 8-pole Bessel), digitized at 4–10 kHz, and stored on a Pentium microcomputer. Holding current (0.00 to ~0.48 pA) was monitored and cells were discarded if this value changed by >25% during perfusion with hyposmotic solutions.

Whole cell series resistance was also measured before (10.6 ± 0.7
MΩ) and after (10.9 ± 0.8 MΩ) the addition of hypotonic solutions; no significant difference in series resistance was observed (P = 0.94; Student’s t-test). EPSC events were analyzed individually by using a floating cursor in Axoscope (Axon); amplitude, duration, and frequency measurements were made from recording sweeps of 30–170 s in duration. For each mossy cell, at least 100 individual EPSC events were analyzed.

RESULTS

Whole cell voltage-clamp recordings were obtained from visually identified O/A interneurons, hilar interneurons, and mossy cells. All neurons displayed prominent voltage-activated K+ currents during cell depolarization in normosmolar recording medium containing 1 μM tetrodotoxin (to block Na+ channels) and 100 μM cadmium (to block Ca2+ channels). Hypomolar external solutions produced a striking and reversible potentiation of voltage-activated K+ currents recorded from hilar and O/A interneurons (Fig. 1). We measured and compared the effect of hypomolar challenge on the amplitudes of the delayed rectifier K+ current (I_k) for all three cell types. A significant increase in I_k amplitude was observed for O/A and hilar interneurons but not mossy cells (Fig. 1A); no change in estimated whole cell capacitance was observed for O/A interneurons (normosmolar: 16 ± 2 pF; hypomolar: 15 ± 2 pF; P = 0.75, Student’s t-test). A prominent, fast transient K+ current (I_ks) was observed on O/A interneurons (Fig. 1B), as described previously (Zhang and McBain 1995). This peak was potentiated by 52% during application of hypomolar solutions (Fig. 1C); no change in I_ks time-to-peak was observed (normosmolar: 8 ± 3 ms; hypomolar: 8 ± 2 ms; P = 0.84). We also observed a 42% potentiation of I_ks time-to-peak during application of hypomolar solutions to hilar interneurons (normosmolar: 8 ± 2 ms; hypomolar: 7 ± 1 ms; P = 0.39); I_ks was not observed on mossy cells. To investigate whether pharmacological blockade of mechanosensitive channels prevented the potentiation of K+ current, we tested the effects of gadolinium (Gd3+), Gd3+ (20–100 μM; ED50 25 μM) has been shown to block mechanosensitive ion channels in other neurons (Oliet and Bourque 1996; Swerup et al. 1991). Hypomolar solution containing 25 μM Gd3+ failed to prevent potentiation of K+ current recorded from O/A interneurons (I_k increased by 60%). At concentrations of 30 and 100 μM, Gd3+ produced a large (>75%), nonspecific, (i.e., also seen in normosmolar solution) and reversible reduction in outward currents (n = 4).

In the same experiments in which no change in K+ currents was observed in mossy cells, spontaneous synaptic currents (3–15 pA) were monitored. Polarity (inward currents with holding potential near ECl) and voltage dependence (increased with hyperpolarized holding potentials) identified these currents as spontaneous EPSCs. Hypomolar solutions elicited large amplitude (10–45 pA) EPSCs on all mossy cells tested (Fig. 2; n = 10); no qualitative changes in EPSCs were observed for O/A or hilar interneurons. Mossy cell EPSCs remained potentiated for the duration of the recording in hypotonic media (30–45 min) and were not reduced by raising cadmium to a saturating concentration of 200 μM (n = 2). Mossy cells (n = 3) in which the holding current (holding potential, −60 mV) did not fluctuate by more than 0.1 pA during the >30 min recording period were chosen for quantitative analysis. Hypomolar solutions produced significant changes in EPSC amplitude and duration; no changes in EPSC frequency were observed (Table 1). Mossy cell EPSCs recorded in hypotonic media were completely blocked by the addition of a glutamate receptor antagonist (10 μM CNQX) to the bathing solution (n = 4; Fig. 3).

DISCUSSION

We have found that a reduction in extracellular osmolarity selectively potentiates voltage-activated K+ currents recorded from inhibitory interneurons and enhances spontaneous...
Neurons synthesize and release specific neurotransmitters onto many different receptors, which permits a degree of selectivity in modulatory actions. In contrast, nonsynaptic interactions (e.g., ephaptic interactions, electrical field effects, or fluctuations in extracellular osmolarity) are believed to modulate neuronal activity in a more nonspecific manner. However, our data argue for a cell-specific selectivity in the effects of osmolarity on neuronal function. In whole cell and single-channel recordings, we initially found that osmotic
TABLE 1. Effects of osmolarity on mossy cell EPSCs

<table>
<thead>
<tr>
<th>Cell</th>
<th>Normosmolar</th>
<th>Hyposmolar</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Cell 1</td>
<td>6.7 ± 0.5</td>
<td>12.2 ± 0.5</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>40 ± 1</td>
<td>97 ± 10</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>1.7</td>
<td>NA</td>
</tr>
<tr>
<td>Cell 2</td>
<td>6.6 ± 0.3</td>
<td>9.4 ± 0.4</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>21 ± 1</td>
<td>25 ± 2</td>
<td>P = 0.0001*</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>1.9</td>
<td>NA</td>
</tr>
<tr>
<td>Cell 3</td>
<td>6.8 ± 0.2</td>
<td>11.0 ± 0.6</td>
<td>P &lt; 0.0001*</td>
</tr>
<tr>
<td></td>
<td>28 ± 1</td>
<td>30 ± 1</td>
<td>P = 0.082</td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>2.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Data were analyzed using a Kolgorov-Smirnov normality test followed by a Wilcoxon signed rank test for repeated measures. EPSCs, excitatory postsynaptic currents; NA, not applicable. *Significance was taken as P < 0.05.

stress potentiated K+ channel function and reduced firing frequency of L/M interneurons, but had no effect on CA1 and subicular pyramidal neurons (Baraban et al. 1997). In the present study we present additional evidence that changes in extracellular osmolarity selectively modulate K+ channel function of O/A and hilar inhibitory (GABAergic) interneurons whereas glutamatergic mossy cells are not affected.

This osmotic mechanism differs from a similar type of interaction seen in other preparations where modulation of ion channel activity is induced by mechanical membrane stretch (Sach 1992). Gd3+, a commonly used pharmacological mechanoreceptor antagonist, failed to prevent osmolarity-induced potentiation of K+ current in our studies. The lack of Gd3+ blockade and the voltage-dependency of osmosensitive K+ channels distinguishes our results from previously described mechanosensitive mechanisms.

Furthermore, this osmolarity-induced modulation of interneuron K+ current differs from recent reports of low osmolarity effects on CA1 pyramidal neurons (Azouz et al. 1997; Huang et al. 1997). Studies using low [NaCl] solutions (Huang et al. 1997) reported an increase in whole cell capacitance on CA1 pyramidal neurons, and Azouz et al. (1997) demonstrated an increase in membrane time constant and modulation of endogenous burst firing of CA1 neurons. We observed no changes in capacitance during perfusion with hypsomotic media, consistent with a report by Langton (1993). Our previous experiments showed that the K+ channel activity of CA1 neurons was not altered by changes in osmolarity (Baraban et al. 1997). These differences may be explained by our focus on isolated potassium currents (I_K and I_A), which involved blocking voltage-dependent sodium currents with tetrodotoxin and testing equi-[NaCl] solutions in which osmolarity was altered by the addition of sucrose. In both the Huang et al. (1997) and Azouz et al. (1997) studies, investigators focused on hypsomolar modulation of a persistent sodium current (I_{Na_p}), but changed osmolarity by lowering extracellular [NaCl]. Additional experiments are needed to determine whether Na+ channel activity is osmosensitive under conditions of a constant extracellular [NaCl].

It is clear that osmotic influences can be seen at the synaptic level as well as at the level of voltage-dependent currents in the soma-dendritic membrane. Strowbridge and Schwartzkroin (1996) suggested that mossy cell excitatory postsynaptic potentials (or EPSCs) could be modulated retrogradely by changes in postsynaptic intracellular calcium concentration ([Ca2+]i). Somjen et al. have shown (1997) that baseline [Ca2+]i levels in hippocampal neurons are initially depressed and then enhanced by exposure to hypsomotic media. However, our recording conditions argue against postsynaptic calcium changes as the determinant of the mossy cell EPSC potentiation observed in our studies. First, [Ca2+]i responses would be dampened by the high concentration of EGTA in the patch pipette. Second, blockade of voltage-activated Ca2+ channels with cadmium did not change the hypsomotic-induced potentiation of mossy cell EPSCs. Third, osmotically induced changes in [Ca2+]i would be expected to occur in a non-neuron-specific manner, yet we only observed enhancement of EPSCs for mossy cells and not for O/A and hilar interneurons. Although the mechanism by which osmolarity modulates spontaneous EPSCs (this study), stimulation-evoked EPSCs (Huang et al. 1997), and NMDA-induced currents (Paoletti and Ascherterneuron KEPSC) (1994) is not presently known, these findings suggest that osmotic changes can play an important role in regulation of hippocampal synaptic activity as well as membrane excitability.

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![FIG. 3. Effect of 6-cyano-7-nitroquin-oxaline-2,3-dione (CNQX) on mossy cell EPSCs. Representative whole cell voltage-clamp recording from a mossy cell during recording in normosmolar media and ~20 min after application of hypsomolar solution containing 10 μM CNQX. Calibration bars: 10 nV and 4 s (top); 10 nV and 100 ms (bottom). Currents were recorded at a holding potential of −55 mV.](image-url)
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