Osmolarity modulates K^+ channel function on rat hippocampal interneurons but not CA1 pyramidal neurons

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1. Whole-cell and single-channel recording methods were used in conjunction with infrared video microscopy techniques to examine the properties of voltage-activated potassium channels in hippocampal neurons during the application of hyposmolar solutions to hippocampal slices from rats.

2. Hyposmolar external solutions (osmolarity reduced by 10% to 267 mosmol l^{-1}) produced a significant potentiation of voltage-activated K^+ current on lacunosum/moleculare (L/M) hippocampal interneurons, but not on CA1 and subiculum pyramidal neurons. Hyperpolarization-activated (I_H) and leak currents were not altered during the application of hyposmolar solutions in all cell types.

3. Mean channel open time and the probability of channel opening were dramatically increased under hyposmolar recording conditions for outside-out patches from L/M interneurons; no changes were observed for patches from CA1 pyramidal neurons. Mean current amplitude and the threshold for channel activation were not affected by hyposmotic challenge.

4. Hyposmolar external solutions produced a significant reduction in the firing frequency of L/M interneurons recorded in current-clamp mode. Hyposmolar solutions had no effect on resting membrane potential, action potential amplitude or duration, and spike after-hyperpolarization amplitude.

5. These results indicate that selective modulation of interneuron ion channel activity may be a critical mechanism by which osmolarity can regulate excitability in the central nervous system.

A reduction in extracellular osmolarity promotes epileptiform activity both clinically and in the hippocampal slice preparation (Andrew, 1991; Ballyk, Quackenbush & Andrew, 1991). It has been proposed that the reduction in extracellular space (ECS) that occurs during periods of hyposmotic stress promotes neuronal synchronization and hyperexcitability via non-synaptic mechanisms. For example, decreasing the ECS may exaggerate the effects of fluctuations in extracellular ion concentrations and increase extracellular electrical resistance (Andrew, 1991; Traub, Dudek, Snow & Knowles, 1985). However, it is not known whether a change in the extracellular environment (e.g. a change in extracellular osmolarity) has a direct effect on ion channel function. If such modulation occurred, it would serve as an important non-synaptic mechanism for regulating neuronal excitability under both normal and pathophysiological conditions.

Cell swelling, which occurs with reduced osmolarity of the extracellular medium, has been shown to activate stretch-sensitive (or inactivate stretch-inactivated) ion channels in cultured or acutely dissociated cell preparations (Guharay & Sachs, 1984; Morris & Sigurdson, 1989; Kim & Fu, 1993; Oliet & Bourque, 1996). Ligand-gated *N*-methyl-D-aspartate channels and voltage-activated calcium channels in cultured neurons are also sensitive to changes in osmolarity (Langton, 1993; Paoletti & Ascher, 1994). In non-neuronal cells, voltage-activated K^+ currents are influenced by mechanical changes (either directly or indirectly) mediated by osmotic stress (Schoenmakers, Vaudry & Cazin, 1995). It has been hypothesized that hyposmolar solutions produce a mechanical stretch of channel proteins, opening the pore to permit a greater influx of ions. This mechanical stretch hypothesis (Morris, 1990; Sachs, 1992; Paoletti & Ascher, 1994) also predicts that osmotic stress would increase voltage-activated ion currents in a neuronally non-specific manner, i.e. on all cell types. Since measurements of channel function in response to osmotic stimulation have been made exclusively on cultured or acutely dissociated cells, it is not known whether changes in extracellular osmolarity modulate ion channel function on neurons maintained in a system that more closely reflects *in vivo* conditions (i.e. with a 'normal' extracellular environment).

We investigated this issue in the hippocampal slice preparation, where cell-to-cell interactions and the extracellular space relationships are generally maintained.

METHODS

Hippocampal slice preparation

Transverse hippocampal slices (300 μm thick) were prepared from 15- to 25-day-old Sprague–Dawley rats, as previously described (Baraban & Schwartzkroin, 1996). Briefly, rats were decapitated, the brain was rapidly removed and chilled briefly in ice-cold, oxygenated (95% O_2–5% CO_2) sucrose artificial cerebrospinal fluid consisting of (mM): 220 sucrose, 3 KCl, 1·25 NaH_2PO_4, 1·2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$ and 10 dextrose (295–305 mosmol l^{-1}; measured on an osmometer). The substitution of sucrose for NaCl during preparation yields slices with greater numbers of viable neurons (Aghajanian & Rasmussen, 1989; Richerson & Messer, 1995). The brain was then blocked and glued to the stage of a Vibroslicer (Frederick Haer, Brunswick, ME, USA) and slices cut from the temporal half of the hippocampus in oxygenated sucrose artificial cerebrospinal fluid at 4 °C. The resulting slices were then transferred to a holding chamber, where they were submerged in oxygenated normal artificial cerebrospinal fluid consisting of (mM): 124 NaCl, 3 KCl, 1·25 NaH_2PO_4, 1·2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$ and 10 dextrose (295–305 mosmol l^{-1}). Slices were held at room temperature for at least 45 min and experiments performed within 8 h. Slices were perfused with extracellular solution at a rate of ~2 ml min^{-1}. All recordings were made at room temperature (22–24 °C).

Whole-cell recording

Tight-seal (4–16 GΩ) whole-cell voltage-clamp recordings were made with an Axopatch-1D amplifier (Axon Instruments) with appropriate series and capacitance compensation (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Tight-seal whole-cell current-clamp recordings were made with an Axoclamp-2A amplifier (Axon Instruments). Patch pipettes were pulled from 1·5 mm borosilicate filament-containing glass tubing (Warner Instrument Corp., Hamden, CT, USA) using a two-stage process, fire-polished and coated with Sylgard® (Dow Corning). The pipette was positioned under visual control using a Zeiss Axioscope and water-immersion (×40) objective with Nomarski differential interference contrast optics and an infrared camera (Hammatsu Co., Hamamatsu, Japan). The command protocol to activate voltage-dependent K^+ currents involved a hyperpolarizing prepulse to −115 mV, followed by six depolarizing voltage steps (20 mV increments) from the holding potential (−60 mV); a hyperpolarizing pulse following the command potentials was used to monitor cell capacitance and series resistance. Currents were leak subtracted on-line using a $P/4$ protocol. Voltage-clamp command potentials and analysis of currents were performed using pCLAMP software (Axon Instruments). Current records were low-pass filtered at 1–2 kHz (−3 dB, 8-pole Bessel), digitized at 4–10 kHz using a Digidata 1200 A/D interface, and stored on a IBM-compatible 486 microcomputer.

Single-channel recording

Electrophysiological investigations were performed on excised membrane patches in outside-out configuration with an Axopatch-1D amplifier (Hamill *et al.* 1981). Patch holding potential was −60 mV. To obtain ensemble currents the command potential was stepped to −115 mV (15 ms) followed by a depolarizing voltage step to +40 mV. For recording steady-state currents, the holding potentials ranged from −60 to +20 mV for durations of 60–300 s. Single-channel recordings were digitally low-pass filtered (−3 dB, 8-pole Bessel) with a cut-off frequency of 0·5 kHz and sampled at 2 kHz. The data were stored on videotape and analysed off-line using pCLAMP software. The single-channel events were measured by 50% threshold detection and verified individually in Fetchan (Axon Instruments). The current amplitude histograms were fitted with Gaussian functions and the decay of the open time histograms with single- or bi-exponential functions.

Solutions

Normosmolar extracellular solution (297 mosmol l^{-1}) consisted (mM) of either: (i) 124 NaCl, 3 KCl, 1·25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$ and 10 dextrose; or (ii) 104 NaCl, 3 KCl, 1·25 NaH_2PO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 2 $CaCl_2$ and 10 dextrose with the addition of sucrose (~32 g l^{-1}). Hyposmolar solutions (267 mosmol l^{-1}) were made by the reduction of [NaCl] (from 124 to 104 mM); hyperosmolar solutions (327 mosmol l^{-1}) contained 104 mM NaCl plus 65 mM sucrose. All solutions were adjusted to pH 7·4 and bubbled with 95% O_2–5% CO_2. Patch pipettes were filled with internal recording solution consisting of (mM): 160 KCH_3SO_4, 10 Hepes, 11 EGTA, 1 $CaCl_2$, 1 $MgCl_2$, 4 Na-ATP and 0·4 Na-GTP (pH was adjusted to 7·25 with 10 M KOH and osmolarity adjusted to 285–290 mosmol l^{-1} with sucrose). Intracellular Ca^{2+} was buffered by 11 mM EGTA to reduce the potential effects of cell swelling-induced increases in intracellular free Ca^{2+} concentration (Wong & Chase, 1986; Busch, Varnum, Adelman & North, 1992). The osmolarity of all solutions was measured using a Wescor 5100B vapour pressure osmometer (Wescor Inc., Logan, UT, USA). Pipette solutions were filtered through a 2 μm filter (Millipore). Solutions with different osmolarities were applied to the slice or outside-out membrane patches via rapid bath perfusion (~2 ml min^{-1}).

Statistics

Results are presented as mean values ± S.E.M. Data were analysed using Student's paired t test on the SigmaStat program (Jandel Scientific, San Rafael, CA, USA). Significance level was taken as $P < 0·05$, unless otherwise indicated.

RESULTS

Pharmacological characterization of whole-cell voltage-activated potassium current

Infrared video microscopy in conjunction with patch-clamp recording was used to visualize and monitor lacunosum/moleculare (L/M) interneurons and CA1 pyramidal neurons in hippocampal slices. In whole-cell recordings, L/M interneurons and CA1 pyramidal cells displayed prominent voltage-activated outward currents during cell depolarization in normosmolar bathing medium (297 mosmol l^{-1}) containing tetrodotoxin (1 μM TTX; to block voltage-dependent Na^+ channels) and cadmium (100 μM $CdCl_2$; to block Ca^{2+} channels). In both cell types, application of tetraethylammonium chloride (2·5–20 mM TEA) reduced the sustained, delayed rectifier K^+ currents (presumably I_K) in a dose-dependent manner. TEA blockade of I_K was reversible. At a concentration of 20 mM TEA, I_K was reduced by 74% in L/M interneurons ($n = 4$) and by 64% in CA1 neurons ($n = 3$). In both types of neurons, a prominent fast, transient K^+ current (presumably I_A) was observed during TEA application.

Effects of osmolarity on whole-cell voltage-activated potassium current

When the slice was bathed in hyposmotic solution (osmolarity reduced by 10% to 267 mosmol l^{-1}) we observed comparable increases in cell volume in both types of neurons (Fig. 1*C* and *D*). The soma length and diameter were measured from frame-grabber images with the aid of a digitizing tablet. The soma volume was calculated using the formula for a prolate spheroid, $\pi l d^2/6$, where d is diameter and l is length (Cameron, Averill & Berger, 1983). These measurements were made at the cell soma and allow for an approximation only of soma volume changes; the changes in dendritic volumes may be significantly different. Our measure of cell swelling under hyposmolar conditions indicated a 55% increase in soma volume for L/M interneurons ($n = 3$) and a 43% increase in CA1 pyramidal cells ($n = 4$).

Hyposmolar external solutions produced a significant potentiation of the sustained, delayed rectifier K^+ currents recorded from L/M interneurons ($n = 10$; Fig. 1*Aa* and *Ab*), but did not potentiate voltage-activated K^+ currents on CA1 pyramidal neurons ($n = 8$; Fig. 1*Ba–Bc*). Potentiation

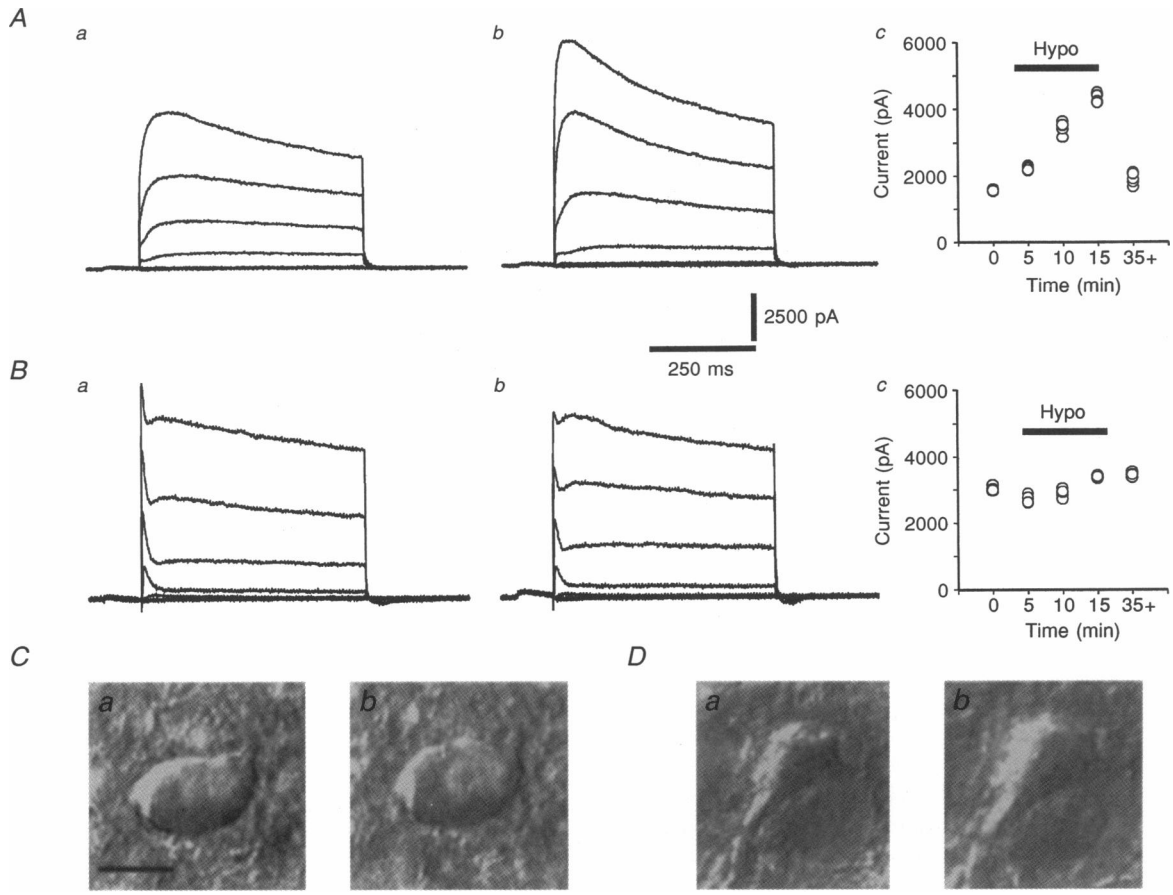

Figure 1. Voltage-activated potassium current is sensitive to changes in extracellular osmolarity: whole-cell recordings

A, whole-cell voltage-clamp recording from an L/M interneuron in normosmolar bathing medium (297 mosmol l^{-1}; *Aa*) and following application of hyposmotic bathing medium (osmolarity reduced by 10% to 267 mosmol l^{-1}; 10 min; *Ab*). Note the osmolarity-mediated increase in K^+ current. *Ac*, plot of the current amplitude measured at the 450 ms time point of a 500 ms depolarizing step to +40 mV. Six measurements were obtained for each time point (○); application of hyposmolar solution (bar) increased I_K amplitudes. *B*, whole-cell voltage-clamp recording from a CA1 pyramidal neuron in normosmolar bathing medium (297 mosmol l^{-1}; *Ba*) and following application of hyposmotic bathing medium (267 mosmol l^{-1}; 10 min; *Bb*). *Bc*, plot of current amplitude *vs.* time for the CA1 neuron, showing no significant change in current amplitude during hyposmolar challenge. *C*, frame-grabber images of an L/M interneuron in normosmolar bathing medium (*Ca*) and following application of hyposmolar solution (measured ~20 min following hyposmolar solution application; *Cb*). *D*, frame-grabber images of a CA1 pyramidal neuron in normosmolar bathing medium (*Da*) and following application of hyposmolar solution (~20 min; *Db*). For both cell types, the hyposmolar challenges caused cell swelling that was reversible when normosmotic solution was restored. Scale bar (in *Ca*), 10 μm.

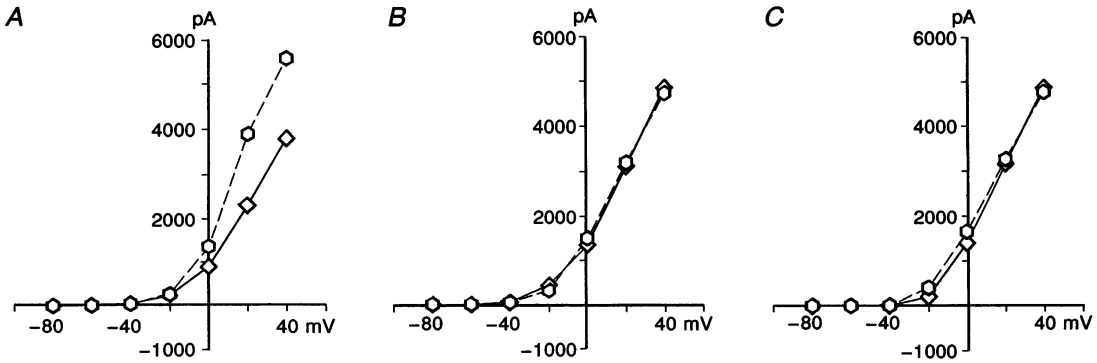

Figure 2. Current–voltage relationship for hippocampal neurons exposed to hyposmolar bathing medium

A, representative current–voltage plot for an L/M interneuron. *B*, representative plot for a CA1 pyramidal neuron. *C*, representative plot for a subiculum neuron. Normosmolar recording conditions (◇; continuous lines); hyposmolar recording conditions (○; dashed lines) are shown. Note that only the L/M interneurons showed a change in the *I–V* relationship following exposure to hyposmolar conditions.

of interneuron K^+ current was initially observed at 2–4 min following application of hyposmolar solution to the slice, reached a maximum at ~15 min, was sustained for recording periods of > 60 min, and recovered to baseline current amplitude during return to normosmolar bathing medium (Fig. 1*A c*). We measured and compared I_K amplitudes in the whole-cell mode, since this current is stable over long periods of whole-cell recording. The amplitude of I_K, measured at the 450 ms time point of a 500 ms depolarizing command, was significantly increased (+51%) by hyposmolar bathing solutions only in L/M interneurons (control, 4107 ± 451 pA; hyposmolar, 5882 ± 513 pA; $n = 10$; $P < 0{\cdot}01$); no significant change (−6%) was observed in I_K amplitude for CA1 pyramidal neurons (control, 3509 ± 509 pA; hyposmolar, 3323 ± 423 pA; $n = 8$, $P = 0{\cdot}46$). The fast, transient K^+ current also increased during application of hyposmotic solutions but was not quantified owing to heterogeneity of I_A across L/M interneurons (not shown). This observation of I_A variability among L/M interneurons is consistent with previous reports of L/M heterogeneity revealed by morphological and sharp intracellular current-clamp recordings (Lacaille & Schwartzkroin, 1988). Although Fig. 1*B* suggests a decrease in I_A on this CA1 pyramidal neuron during the application

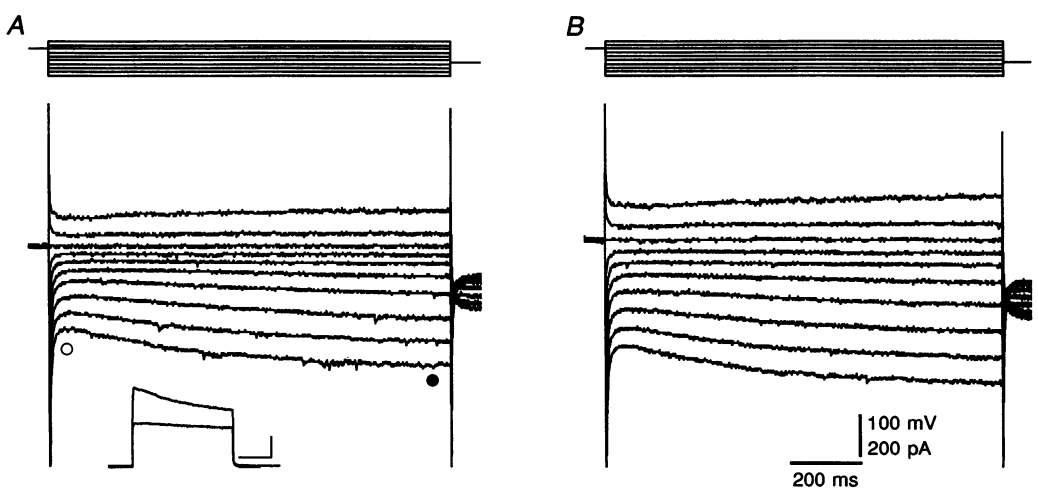

Figure 3. Hyperpolarization-activated currents are not modulated by changes in extracellular osmolarity

A, whole-cell currents (bottom traces) of an L/M interneuron during a series of long hyperpolarizing voltage steps (top traces) under normosmolar recording conditions. *B*, same cell approximately 25 min after application of hyposmolar solution. I_H is not leak subtracted; peak current is determined as the difference between instantaneous (○) and steady-state currents (●). Voltage-activated K^+ current for this neuron is shown in inset in *A* (scale bars: 2500 pA, 200 ms) before (bottom trace) and after application of hyposmolar solution (top trace).

of hyposmolar solutions, this effect was not seen in other CA1 pyramidal cells; measurement of this current revealed a non-significant 10% decrease in current amplitude among pyramidal cells ($n = 4$; $P = 0{\cdot}68$). Hyperosmotic solutions (osmolarity increased by 10% to 327 mosmol l^{-1}) reduced K^+ current by 48% in both L/M interneurons and CA1 pyramidal cells in an irreversible manner ($n = 3$), as previously demonstrated in neuroblastoma × glioma (NG108-15) cells (Schoenmakers *et al.* 1995) and acutely dissociated CA1 pyramidal neurons (Huang, Aitken & Somjen, 1995).

A plot of the steady-state current–voltage relationship (measured at the 450 ms time point of a 500 ms current pulse) was constructed for three representative types of hippocampal neurons: L/M interneuron, CA1 pyramidal neuron and a subiculum pyramidal neuron (Fig. 2). Application of hyposmolar solutions produced a significant potentiation of voltage-activated K^+ current on L/M interneurons at depolarizing steps between +60 and +100 mV; no change in the threshold for outward current activation was observed. Outward current amplitude on CA1

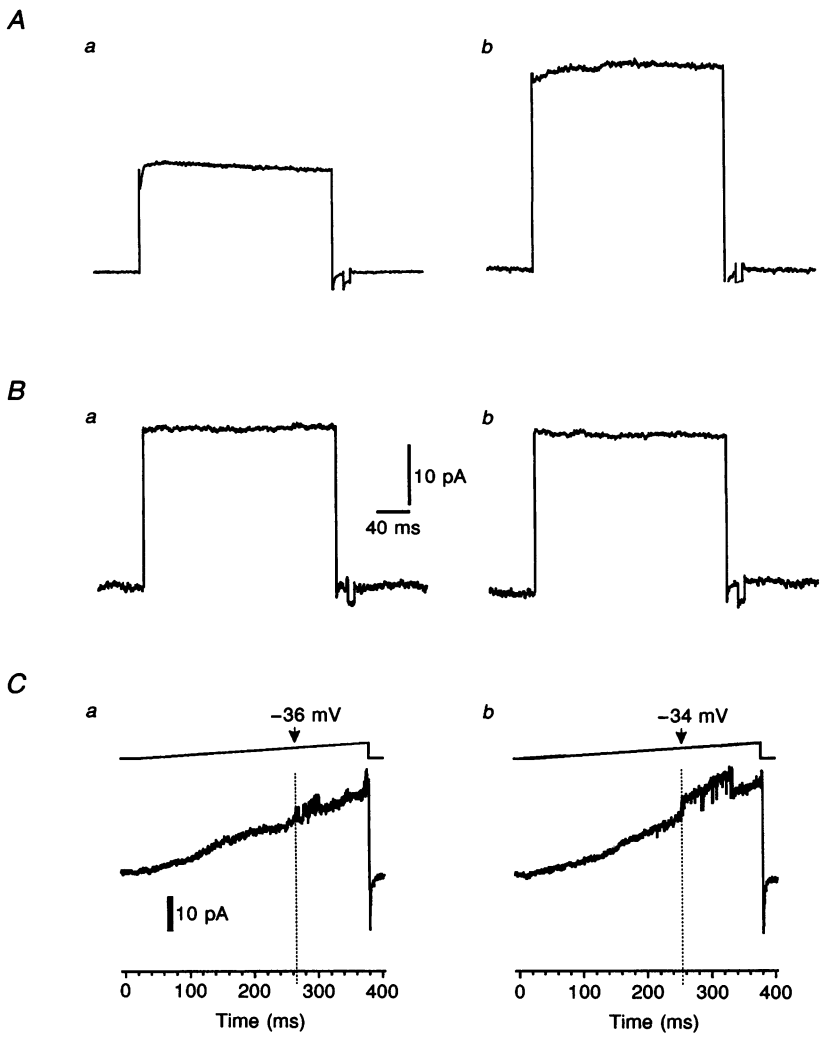

Figure 4. Voltage-activated potassium channels are modulated by extracellular osmolarity: single-channel outside-out patch recordings

Outside-out patches were held at a membrane potential of −60 mV and depolarized (following a hyperpolarizing prepulse) with a protocol designed to match that used in whole-cell experiments; 10–15 current traces were summed to obtain an ensemble current. *A*, average ensemble current obtained during a depolarizing voltage step to +40 mV (following a prepulse hyperpolarization); holding potential, −60 mV. Ensemble average from 15 such trials in normosmolar bathing medium (*A a*) and following application of hyposmolar solution (*A b*; ~10 min). Currents are leak subtracted. *B*, same protocol for a single-channel patch recording from a CA1 pyramidal neuron in normosmolar bathing medium (*B a*) and following application of hyposmolar solution (*B b*; ~10 min). *C*, voltage-ramp protocol (top; −100 to +20 mV) to activate K^+ channels on an L/M interneuron patch under normosmolar recording conditions (297 mosmol l^{-1}; *C a*) and during application of hyposmolar solution (267 mosmol l^{-1}; 15 min; *C b*). Note the osmolarity-mediated increase in channel open time in the absence of a change in threshold for activation.

pyramidal neurons and subiculum pyramidal neurons (control, 2918 ± 393 pA; hyposmolar, 2548 ± 538 pA; $n = 3$; $P = 0{\cdot}61$) were not altered by application of hyposmolar solutions (Fig. 2*B* and *C*).

To test the selectivity of hyposmotic effects on I_K, we also examined osmolarity influences on a hyperpolarization-activated current (Fig. 3). Long hyperpolarizing voltage commands (2 s; Fig. 3, top set of traces) activated a slowly developing inward current (presumably I_H) in both types of neurons. Hyposmolar solutions did not significantly alter the peak amplitude of this current in L/M interneurons (+15%; $n = 6$; $P = 0{\cdot}77$) or in CA1 pyramidal neurons (+4%; $n = 3$; $P = 0{\cdot}84$). Although I_H was not altered during the application of hyposmolar solutions to L/M interneurons, potentiation of I_K could still be observed (see

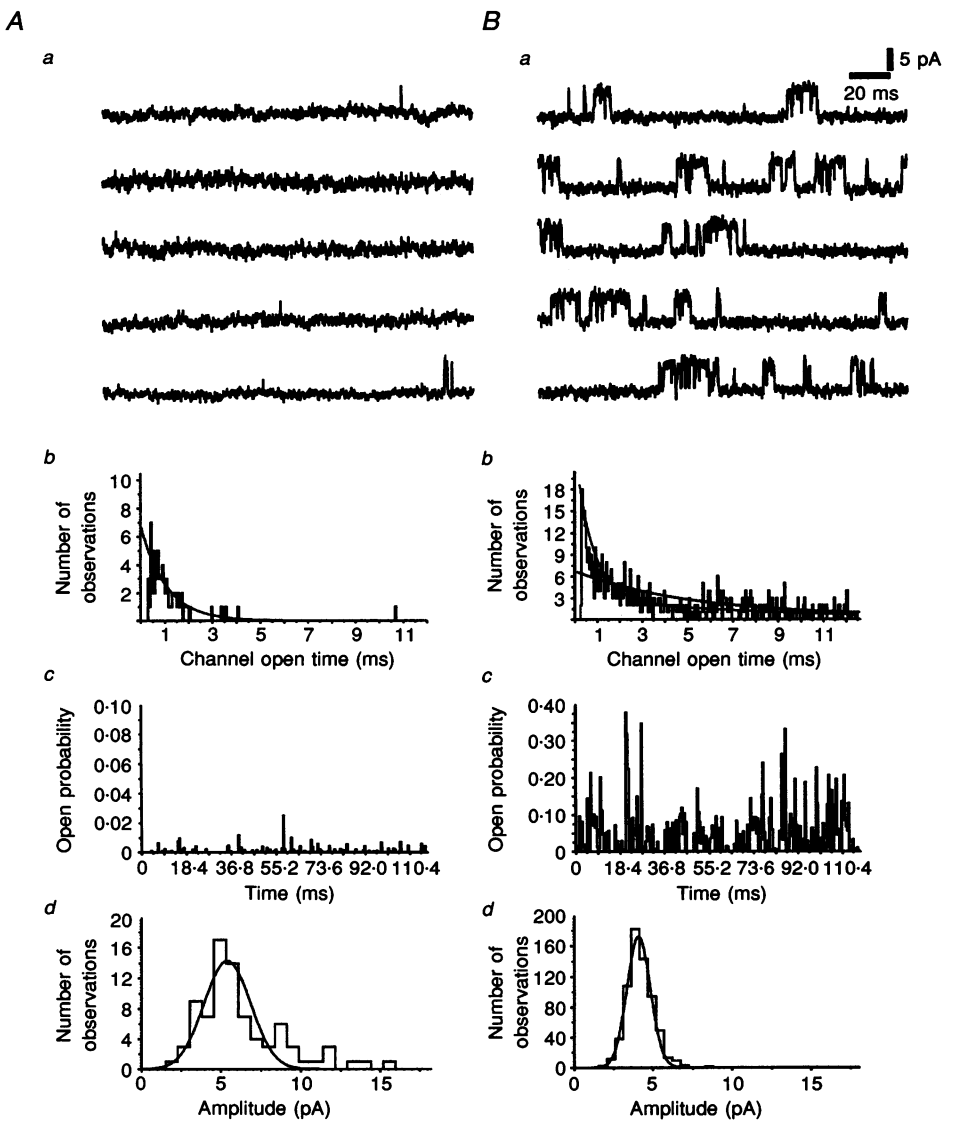

Figure 5. Kinetics of single-channel currents from L/M interneurons

Steady-state single-channel recordings from a representative L/M interneuron patch under normosmolar recording conditions (*A a*, open probability = 0·001) and 15 min following application of hyposmolar solution (*B a*, open probability = 0·113). Currents were elicited at a holding potential of +20 mV. Note the osmolarity-mediated increase in channel activity. Kinetics of single-channel K^+ currents were measured at a steady-state holding potential of +20 mV (120 s recording) during baseline (297 mosmol l^{-1}) and hyposmolar recording conditions (267 mosmol l^{-1}). Histograms of the channel open time were computed for baseline (*A b*) and hyposmolar recording conditions (*B b*). A single exponential function fitted the decay of the histogram in *A b*, but two exponentials were needed to fit the decay in *B b*, indicating a significant difference in channel opening between normosmolar and hyposmolar states. Histograms of the probability of single-channel openings during normosmolar (*A c*) and hyposmolar recordings (*B c*) were also significantly different. However, amplitude histograms of single-channel K^+ currents under normosmolar (*A d*) and hyposmolar recording conditions (*B d*), fitted with Gaussian functions, showed the same mean amplitudes for normo- and hyposmolar conditions.

Table 1. Single-channel potassium currents

Cell type	Normosmolar (297 mosmol l^{-1})	Hyposmolar (267 mosmol l^{-1})
L/M interneuron ($n = 6$)		
Open time (ms)		
τ_0	1·57 ± 0·24	0·69 ± 0·05*
	(1·08–2·5)	(0·44–0·79)
τ_1	—	5·4 ± 0·9*
	—	(2·81–8·72)
Open probability	0·002 ± 0·0007	0·123 ± 0·03*
	(0·001–0·014)	(0·05–0·242)
Amplitude (pA)	4·9 ± 0·6	5·1 ± 0·9
	(3·1–6·7)	(3·4–8·7)
CA1 pyramidal neuron ($n = 3$)		
Open time (ms)		
τ_0	1·09 ± 0·16	1·33 ± 0·19
	(0·87–1·4)	(0·96–1·4)
Open probability	0·11 ± 0·001	0·17 ± 0·007
	(0·01–0·012)	(0·016–0·017)
Amplitude (pA)	4·2 ± 0·15	4·8 ± 0·05
	(4·0–4·3)	(4·7–4·8)

Currents were measured at a steady-state holding potential of +20 mV (120 s recording period). The values represent means ± S.E.M. Range of values is represented in parentheses. * Statistically significant differences from baseline values using Student's paired t test ($P < 0·05$).

Fig. 3*A*, inset). Further, we measured the amplitude of L/M interneuron leak current before and after the application of hyposmolar solutions. Leak current accounted for < 1% of whole-cell voltage-activated current in L/M interneurons and did not change by more than 10 pA during the application of hyposmolar solutions. These experiments demonstrate a neuron- and current-specific modulation of K^+ channel function in the hippocampus during periods of osmolarity-induced cell swelling.

Effect of osmolarity on single-channel voltage-activated potassium current

Single-channel currents were studied in outside-out patches pulled from visually identified L/M interneurons and CA1 pyramidal neurons. In these experiments, we measured and compared the amplitude of ensemble I_K, at the 450 ms time point of a 500 ms depolarizing command. This current was significantly potentiated by hyposmolar bathing solutions in L/M interneurons (60 ± 10%; $n = 7$; $P < 0·05$; Fig. 4*A*); no change was observed in ensemble I_K amplitude for CA1 pyramidal neurons (1 ± 12%; $n = 3$; Fig. 4*B*). Slow voltage-ramp protocols (−100 to +20 mV; 350 ms duration) were used to test whether osmolarity-induced changes in K^+ ion channel function could be ascribed to a shift in the threshold for channel activation. Hyposmolar-mediated changes in K^+ channel activity were observed at holding potentials between −25 and +40 mV, and were not associated with a significant change in the threshold for channel activation (Fig. 4*C*; control, −25·3 ± 4·8 mV; range, −12·4 to −41·2 mV; hyposmolar, −25·4 ± 5·1 mV; range, −12·7 to −41·5 mV; $n = 6$; $P = 0·98$). Single-channel voltage-activated current was reduced by 52% during 20 mM TEA application ($n = 3$), as expected for a K^+-selective ion channel (Storm, 1990). The apparent difference between whole-cell and outside-out patch sensitivity to TEA may simply reflect an uneven distribution of TEA-selective K^+ channels in these membrane patches, especially since the range of sensitivities (38–70%) is consistent with the sensitivity to TEA seen in whole-cell recording.

A separate protocol was used to examine steady-state single-channel kinetics during changes in osmolarity. Under normosmolar recording conditions (297 mosmol l^{-1} with 1 μM TTX and 100 μM cadmium), the channel open time, the probability of channel opening, and the amplitude of single-channel currents were determined at a holding potential of +20 mV. In L/M interneuron patches held at +20 mV to activate K^+ channels, a significant increase in K^+ channel activity was observed 1–2 min following application of hyposmolar solutions. This increase persisted for recording periods of > 40 min and channel activity recovered to baseline levels upon return to normosmolar bathing medium (Fig. 5*Aa* and *Ba*); no change was observed for patches from CA1 neurons. The mean amplitude of single-channel currents was not affected by hyposmolar solutions and additional channel open states were not observed (Table 1 and Fig. 5*Ba* and *Bd*). However, channel open time for patches from L/M interneurons was dramatically increased during hyposmolar recording conditions (Table 1). In normosmolar bathing medium the mean open time was fitted by a single exponential for patches from L/M

Table 2. Current-clamp properties of L/M interneurons

	Resting membrane potential (mV)	Action potential amplitude (mV)	Action potential duration (ms)	Spike AHP amplitude (mV)	Number of spikes per 500 ms
Normosmolar (297 mosmol l^{-1})	−62·6 ± 1·9 (−54 to −71)	87 ± 1·6 (71–103)	3·8 ± 0·2 (3–5)	13·1 ± 1·4 (5–22)	14·9 ± 1·5 (7–22)
Hyposmolar (267 mosmol l^{-1})	−61·1 ± 1·6 (−52 to −69)	88 ± 2·9 (78–96)	4·2 ± 0·4 (3–6)	10 ± 1·1 (6–16)	4·4 ± 0·8* (1–10)

The values represent means ± S.E.M. ($n = 11$). Range of values is represented in parentheses. AHP, after-hyperpolarization. * Statistically significant difference from baseline values using Student's paired t test ($P < 0·001$).

interneurons (time constant, $\tau_0 = 1·57 \pm 0·24$ ms); this value increased ($\tau_0 = 4·9 \pm 0·9$ ms; $P < 0·05$) following application of hyposmolar recording solutions. Under hyposmolar recording conditions channel open time histograms were best fitted by a bi-exponential equation yielding two time constants, τ_0 and τ_1 (Table 1; compare Fig. 5*A b* and *B b*). The probability of channel opening for patches from L/M interneurons was also altered during application of hyposmolar solutions. These changes in single-channel kinetics were not observed for patches from CA1 pyramidal neurons (Table 1). Therefore, changes in potassium current observed during conditions of hyposmolar-induced cell swelling are selectively mediated by changes in single-channel kinetics.

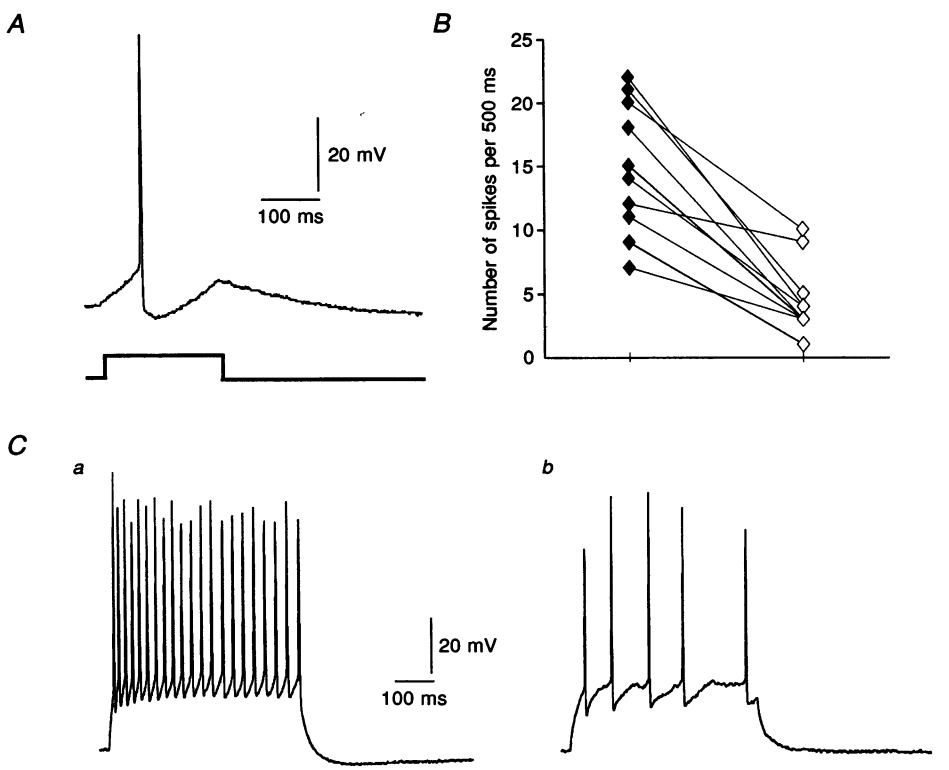

Figure 6. L/M interneuron firing frequency is modulated by extracellular osmolarity

A, representative current-clamp recording of an L/M interneuron in normosmolar solution during application of a 100 ms depolarizing current injection (bottom trace; 0·1 nA for 200 ms). *B*, plot of the firing frequency for all eleven L/M interneurons tested, illustrating the difference in discharge properties under normosmolar conditions (◆) and following application of hyposmolar solutions (~20 min; ◇). *C*, representative current-clamp recording of an L/M interneuron under normosmolar recording conditions (resting membrane potential, −70 mV; *Ca*) and 20 min after application of hyposmolar solution (resting membrane potential, −71 mV; *Cb*). Depolarizing current step is 0·5 nA for 500 ms. Action potential amplitudes clipped by digitizing process.

Effects of osmolarity on the firing properties of L/M interneurons

In a separate set of experiments we used current-clamp recordings to determine whether osmolarity-mediated changes in K^+ channel function were associated with a change in cell firing properties. Eleven visually identified L/M interneurons were recorded using infrared video microscopy in whole-cell current-clamp mode. These cells did not fire action potentials spontaneously and, when caused to fire by intracellular current injection, were characterized by a large spike after-hyperpolarization, as reported previously (Lacaille & Schwartzkroin, 1988; Williams, Samulack, Beaulieu & Lacaille, 1994). A representative recording from one such L/M interneuron is shown in Fig. 6*A*. Application of hyposmolar solutions did not significantly alter intrinsic cell properties such as resting membrane potential, action potential amplitude and duration, and spike after-hyperpolarizations (Table 2), suggesting that voltage-activated sodium and calcium channels are not sensitive to changes in osmolarity. However, hyposmolar solutions did produce a significant modulation of the firing frequency of L/M interneurons. The number of action potentials generated during a long depolarizing step (500 ms, 0·5 nA) was determined for all neurons before and 20 min after application of hyposmolar solutions (Table 2; Fig. 6*B*). Hyposmolar solutions dramatically reduced the firing frequency in these neurons. A representative recording of an L/M interneuron before and after application of hyposmolar solution is shown in Fig. 6*C*. Partial recovery of firing frequency was observed approximately 20 min after return to normosmolar bathing medium. These results suggest that osmotic stress can directly modulate neuronal excitability in the hippocampal slice preparation.

DISCUSSION

In these studies, we observed a selective and neuron-specific modulation of K^+ current during modest changes in osmolarity (30 mosmol l^{-1}). Our data suggest that (i) osmolarity may play a critical role in regulating neuronal excitation during both normal and pathophysiological conditions and (ii) there may be fundamental differences in pyramidal *vs.* interneuron K^+ channel structure, which produce a differential sensitivity to changes in extracellular osmolarity.

Potential mechanism of action for osmosensitive potassium channels

There are a number of potential mechanisms that might contribute to the phenomenon observed in our studies. First, mechanical membrane stretch of ion channels has been described for several cell types (Sackin, 1989; Morris & Sigurdson, 1989) and may contribute to the modulation of ion channel function observed in this study. It has been suggested that these stretch-sensitive ion channels are activated (or inactivated) by membrane tension. In previous studies, stretch-sensitive channels generally did not show much voltage dependence and were difficult to observe in whole-cell recordings (Sackin, 1989; Paoletti & Ascher, 1994). This mechanism seems unlikely given our findings that osmosensitive K^+ channels on L/M interneurons are clearly voltage dependent and could be observed in whole-cell recordings. Second, it is also possible that changes in extracellular Na^+ concentration, with corresponding effects on ion fluxes, could contribute to the modulation of channel function observed in this study. However, we observed the same osmotically mediated potentiation of K^+ current in experiments where extracellular Na^+ concentration did not change. Third, that the observed effect on L/M interneuron potassium channels is osmotically rather than mechanically mediated is suggested by the fact that it would be difficult to produce a significant amount of mechanical stretch in outside-out membrane patches during a 10% change in osmolarity. In previous studies (Sackin, 1989; Ackerman, Wickman & Clapham, 1994; Rees, Vandenberg, Wright, Yoshida & Powell, 1995; Oliet & Bourque, 1996), osmotic challenges sufficient to increase the lateral tension experienced by ion channels embedded in a patch were in the range of 50–200 mosmol l^{-1}. The change in extracellular osmolarity required to produce a modulation of K^+ current in our study was only 30 mosmol l^{-1} and is well within the physiological range of cerebrospinal fluid osmolarity fluctuations (11–50 mosmol l^{-1}) measured in 'normal' patients (Nishimura, Shimuzi, Imanaga, Kubo & Yoshida, 1977; Kurokowa *et al.* 1990).

Furthermore, changes in extracellular osmolarity did not appear to produce a non-specific 'mechanical' stretch of ion channels in our experiments. In whole-cell voltage-clamp recordings, leak current and a hyperpolarization-activated current (I_H) were not significantly altered during the application of hyposmolar solutions to L/M interneurons. In whole-cell current-clamp recordings, action potential properties and calcium-dependent spike after-hyperpolarizations were not altered during the application of hyposmolar solutions. In addition, two distinct types of pyramidal neurons (in CA1 and subiculum) were also not affected by changes in osmolarity *in vitro*. As such, our data suggest a novel, direct and neuron-specific effect of hyposmotic (rather than mechanical) stress on voltage-activated K^+ channels.

Zimmerberg and colleagues recently proposed a model for osmosensitive K^+ channels in the squid giant axon: a 'spring-loaded' K^+ channel comprising a spring between a voltage-dependent gate and an osmotically sensitive channel (Zimmerberg & Parsegian, 1986; Zimmerberg, Bezanilla & Parsegian, 1990). The features of this model are consistent with our observations on L/M interneurons inasmuch as osmotic factors did not affect the voltage dependence of gating (i.e. no shift in the threshold for channel activation), but did directly modulate K^+ channel opening kinetics (i.e. increase in the probability of opening and channel open time). One possible explanation for a cell type-specific osmotically sensitive ion channel could be cell-to-cell

differences in molecular structure. For example, a single amino acid base pair residue could confer osmosensitivity on L/M interneuron potassium channels, in the same way that individual K^+ channel residues are involved in pH sensitivity, gating kinetics and ion selectivity (Busch, Hurst, North, Adelman & Kavanaugh, 1991; McCormack, Lin & Sigworth, 1993; Stanfield, Davies, Shelton, Sutcliffe, Brammar & Conley, 1994; Navaratnam, Escobar, Covarrubias & Oberholtzer, 1995).

Functional significance of an osmosensitive potassium channel

Interneurons play a central role in regulating network excitability in the hippocampus (Miles & Wong, 1987). In the CA1 subfield, L/M interneurons mediate primarily feedforward inhibition to CA1 pyramidal cells (Lacaille & Schwartzkroin, 1988; Williams *et al.* 1994), are directly activated by excitatory synaptic input to the hippocampus, and their influence on pyramidal cells is mediated by synaptically released neurotransmitters and neuromodulators (Alger & Nicoll, 1982). An increase in interneuron K^+ current during periods of cell swelling, without compensatory changes in other interneuron currents (or alteration of pyramidal cell K^+ current) could have profound effects on network excitability. For example, if cell swelling during hypoxic–ischaemic or epileptic episodes produced an increase in interneuron I_K, the firing frequency of L/M interneurons would decrease (as shown in Results); the resultant reduction of inhibition to pyramidal neurons would contribute to the hyperexcitability observed under these conditions (Andrew, 1991; Ballyk *et al.* 1991). Indeed, hyposmolar states (e.g. during hypersecretion of antidiuretic hormone or water intoxication) result in cell swelling, hyperexcitability and an increased incidence of seizures (Andrew, 1991; Roper, Obenaus & Dudek, 1992).

Recent work from our laboratory has demonstrated that furosemide (an agent which reduces cell swelling) can block epileptiform activity in a variety of *in vitro* and *in vivo* models (Hochman, Baraban, Owens & Schwartzkroin, 1995). In these studies, intrinsic optical signals obtained during stimulation-induced epileptiform after-discharge activity indicated a significant degree of cell (neuronal or glial) swelling in the stratum lacunosum/stratum moleculare region of the hippocampus. The cell bodies and dendritic arborizations of L/M interneurons are prominently represented in this hippocampal region (Kunkel, Lacaille & Schwartzkroin, 1988; Lacaille & Schwartzkroin, 1988; Williams *et al.* 1994). Following treatment with furosemide, intrinsic optical signal and stimulation-induced epileptiform after-discharge were abolished (Hochman *et al.* 1995). Thus, direct non-synaptic modulation of L/M interneuron excitability during changes in cell swelling (coincident with a reduction in extracellular space) is one possible mechanism for the anti-epileptiform actions of furosemide and other agents which limit or reverse cell swelling.

Conclusion

We have demonstrated that L/M interneurons possess a unique ability to respond to changes in extracellular osmolarity in the *in vitro* hippocampal slice preparation. In contrast to the predicted non-specific effects of mechanical stretch on ion channel function (Morris, 1990; Paoletti & Ascher, 1994), this effect was only observed on L/M interneurons and was specific for TEA-sensitive voltage-activated potassium channels. The recognition of a neuron-selective action of osmotic stress on voltage-activated potassium channels in the hippocampus provides new insights into non-synaptic mechanisms that may play a significant role in the modulation of neuronal excitability in the central nervous system.

Ackerman, M. J., Wickman, K. D. & Clapham, D. E. (1994). Hypotonicity activates a native chloride current in *Xenopus* oocytes. *Journal of General Physiology* **103**, 153–179.

Aghajanian, G. K. & Rasmussen, K. (1989). Intracellular studies in the facial nucleus illustrating a simple new method for obtaining viable motoneurons in adult rat brain slices. *Synapse* **31**, 331–338.

Alger, B. E. & Nicoll, R. A. (1982). Pharmacological evidence for two kinds of GABA receptor on rat hippocampal pyramidal cells studied *in vitro*. *Journal of Physiology* **328**, 125–141.

Andrew, R. D. (1991). Seizure and acute osmotic change: clinical and neurophysiological aspects. *Journal of the Neurological Sciences* **101**, 7–18.

Ballyk, B. A., Quackenbush, S. J. & Andrew, R. D. (1991). Osmotic effects on the CA1 neuronal population in hippocampal slices with special reference to glucose. *Journal of Neurophysiology* **65**, 1055–1066.

Baraban, S. C. & Schwartzkroin, P. A. (1996). Electrophysiology of CA1 pyramidal neurons in an animal model of neuronal migration disorders: prenatal methylazoxymethanol treatment. *Epilepsy Research* **22**, 145–156.

Busch, A. E., Hurst, R. S., North, R. A., Adelman, J. P. & Kavanaugh, M. P. (1991). Current inactivation involves a histidine residue in the pore of the rat lymphocyte potassium channel RGK5. *Biochemical and Biophysical Research Communications* **179**, 1384–1390.

Busch, A. E., Varnum, M., Adelman, J. P. & North, R. A. (1992). Hypotonic solution increases the slowly activating potassium I_{SK} expressed in *Xenopus* oocytes. *Biochemical and Biophysical Research Communications* **184**, 804–810.

Cameron, W. E., Averill, D. B. & Berger, A. J. (1983). Morphology of cat phrenic motoneurons as revealed by intracellular injection of horseradish peroxidase. *Journal of Comparative Neurology* **219**, 70–80.

Guharay, F. & Sachs, F. (1984). Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *Journal of Physiology* **352**, 685–701.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

Hochman, D. W., Baraban, S. C., Owens, J. W. M. & Schwartzkroin, P. A. (1995). Dissociation of synchronization from hyperexcitability in furosemide blockade of epileptiform activity. *Science* **270**, 99–102.

HUANG, R., AITKEN, P. G. & SOMJEN, G. G. (1995). Hypertonia saves hypoxic neurons and prevents SD-like depolarization. *Society for Neuroscience Abstracts* **21**, 213, 88.10.

KIM, D. & FU, C. (1993). Activation of a nonselective cation channel by swelling in atrial cells. *Journal of Membrane Biology* **135**, 27–37.

KUNKEL, D. D., LACAILLE, J.-C. & SCHWARTZKROIN, P. A. (1988). Ultrastructure of stratum lacunosum-moleculare interneurons of hippocampal CA1 region. *Synapse* **2**, 382–394.

KUROKOWA, Y., SUZUKI, K., HASHI, K., UEDE, T., MATSUMURA, S., KAWAHARA, I., YAJIMA, Y., UJIKE, M. & KANEKO, M. (1990). Elevation of intracranial pressure during hemodialysis – continuous measurement of cerebrospinal fluid pressure in a patient with acoustic neurinoma. *No To Shinkei (Brain and Nerve)* **42**, 569–573.

LACAILLE, J.-C. & SCHWARTZKROIN, P. A. (1988). Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. I. Intracellular response characteristics, synaptic responses, and morphology. *Journal of Neuroscience* **8**, 1400–1410.

LANGTON, P. D. (1993). Calcium channel currents recorded from isolated myocytes of rat basilar artery are stretch sensitive. *Journal of Physiology* **471**, 1–11.

MCCORMACK, K., LIN, L. & SIGWORTH, F. J. (1993). Substitution of a hydrophobic residue alters the conformational stability of *Shaker* K^+ channels during gating and assembly. *Biophysical Journal* **65**, 1740–1748.

MILES, R. & WONG, R. K. S. (1987). Inhibitory control of local excitatory circuits in the guinea-pig hippocampus. *Journal of Physiology* **388**, 611–629.

MORRIS, C. E. (1990). Mechanosensitive ion channels. *Journal of Membrane Biology* **113**, 93–107.

MORRIS, C. E. & SIGURDSON, W. J. (1989). Stretch-inactivated ion channels coexist with stretch-activated ion channels. *Science* **243**, 807–809.

NAVARATNAM, D. S., ESCOBAR, L., COVARRUBIAS, M. & OBERHOLTZER, J. C. (1995). Permeation properties and differential expression across the auditory receptor epithelium of an inward rectifier K^+ channel cloned from chick inner ear. *Journal of Biological Chemistry* **270**, 19238–19245.

NISHIMURA, T., SHIMUZI, T., IMANAGA, H., KUBO, O. & YOSHIDA, S. (1977). Two cases of nonketotic hyperosmolar coma in neurosurgery. *No Shinkei Geka (Neurological Surgery)* **5**, 1165–1170.

OLIET, S. H. R. & BOURQUE, C. W. (1996). Gadolinium uncouples mechanical detection and osmoreceptor potential in supraoptic neurons. *Neuron* **16**, 175–181.

PAOLETTI, P. & ASCHER, P. (1994). Mechanosensitivity of NMDA receptors in cultured mouse central neurons. *Neuron* **13**, 645–655.

REES, S. A., VANDENBERG, J. I., WRIGHT, A. R., YOSHIDA, A. & POWELL, T. (1995). Cell swelling has differential effects on the rapid and slow components of delayed rectifier potassium current in guinea pig cardiac myocytes. *Journal of General Physiology* **106**, 1151–1169.

RICHERSON, G. B. & MESSER, C. (1995). Effect of composition of experimental solutions on neuronal survival during rat brain slicing. *Experimental Neurology* **131**, 133–143.

ROPER, S. N., OBENAUS, A. & DUDEK, F. E. (1992). Osmolality and nonsynaptic epileptiform bursts in rat CA1 and dentate gyrus. *Annals of Neurology* **31**, 81–85.

SACHS, F. (1992). Stretch-sensitive ion channels: an update. In *Sensory Transduction*, ed. COREY, D., pp. 242–260. Rockefeller University Press, New York.

SACKIN, H. (1989). A stretch-activated K^+ channel sensitive to cell volume. *Proceedings of the National Academy of Sciences of the USA* **86**, 1731–1735.

SCHOENMAKERS, T. J. M., VAUDRY, H. & CAZIN, L. (1995). Osmo- and mechanosensitivity of the transient outward K^+ current in a mammalian neuronal cell line. *Journal of Physiology* **489**, 419–430.

STANFIELD, P. R., DAVIES, N. W., SHELTON, P. A., SUTCLIFFE, M. J., KHAN, I. A., BRAMMAR, W. J. & CONLEY, E. C. (1994). A single aspartate residue is involved in both intrinsic gating and blockage of Mg^{2+} of the inward rectifier, IRK1. *Journal of Physiology* **478**, 1–16.

STORM, J. F. (1990). Potassium currents in hippocampal pyramidal cells. *Progress in Brain Research* **83**, 161–187.

TRAUB, R. D., DUDEK, F. E., SNOW, R. W. & KNOWLES, W. D. (1985). Computer simulations indicate that electrical field effects contribute to the shape of the epileptiform field potential. *Neuroscience* **15**, 947–958.

WILLIAMS, S., SAMULACK, D. D., BEAULIEU, C. & LACAILLE, J.-C. (1994). Membrane properties of interneurons located near the stratum lacunosum-moleculare/radiatum border of area CA1 in whole-cell recordings from rat hippocampal slices. *Journal of Neurophysiology* **71**, 2217–2235.

WONG, S. M. E. & CHASE, H. S. JR (1986). Role of intracellular calcium in cellular volume regulation. *American Journal of Physiology* **250**, C841–852.

ZIMMERBERG, J., BEZANILLA, F. & PARSEGIAN, V. A. (1990). Solute inaccessible aqueous volume changes during opening of the potassium channel of the squid giant axon. *Biophysical Journal* **57**, 1049–1064.

ZIMMERBERG, J. & PARSEGIAN, V. A. (1986). Polymer inaccessible volume changes during opening and closing of a voltage-dependent ionic channel. *Nature* **323**, 36–39.

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