GLIA 20:254–261 (1997)

Increased Potassium, Chloride, and Taurine Conductances in Astrocytes During Hypoosmotic Swelling

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KEY WORDS brain edema; patch clamp; cell culture; cell volume

ABSTRACT Membrane conductances during hypoosmotic swelling were characterized in rat astrocytes in primary tissue culture. Using whole cell patch clamp techniques, mean ± SEM cell conductance in isoosmotic phosphate-buffered saline (PBS) was 55.6 ± 5.8 pS/pF. Cell conductance (mean ± SEM) increased from this initial value to 187 ± 46%, 561 ± 188%, and 1216 ± 376% within 9 min of exposure to 220 mOsm, 190 mOsm, and 145 mOsm PBS, respectively. With each of these hypoosmotic exposures, no change occurred in membrane capacitance. When CsCl replaced KCl in the microelectrode solution, a similar conductance increase was obtained at each osmolality. However, when gluconate salts were used in place of chloride salts in the electrode solution, no significant conductance increase was observed with 190 mOsm PBS. With a KCl microelectrode solution, all conductance increase which occurred in 190 mOsm PBS was inhibited by 200 μM niflumic acid, but not by 5 mM $BaCl_2$. Both niflumic acid and $BaCl_2$ inhibited 60–80% of the conductance increase of cells in 145 mOsm PBS. Using a microelectrode solution containing taurine as the major anion, membrane conductance increased 5-fold when cells were placed in 250 mOsm medium. This conductance increase was completely inhibited by 200 μM niflumic acid. Thus, independent chloride and potassium conductances are activated by hypoosmotic swelling of cultured astrocytes while plasma membrane area is unaltered. The chloride conductance pathway is activated at a significantly lower degree of hypoosmotic exposure than that which activates the potassium pathway and may be permeable to anionic taurine. These conductance pathways may mediate diffusive loss of potassium, chloride, and taurine from these cells during volume regulation following hypoosmotic swelling. *GLIA 20:254–261, 1997.*

INTRODUCTION

Cerebral astrocytes from primary culture swell rapidly in hypoosmotic conditions and then demonstrate a regulatory volume decrease over the next 15–30 min due to net reduction in total intracellular osmolyte content (Kimelberg and Frangakis, 1985; Olson et al., 1986). Loss of several intracellular constituents including potassium, taurine, and other amino acids has been associated with this volume regulatory response (Bender and Norenberg, 1994; Kimelberg et al., 1990b; O'Connor and Kimelberg, 1993; Vitarella et al., 1994). The relative contribution of each of these osmolytes to the recovery of normal cell volume depends upon the magnitude of the osmotic challenge. When astrocytes are transferred from normal extracellular conditions (290 mOsm) to solutions with osmolalities of 170 mOsm and higher, no net change in cellular potassium content is observed (Olson and Kimelberg, 1995; Olson et al.,

Contract grant sponsor: Kettering Medical Center; Contract grant sponsor: National Institutes of Health; Contract grant number: NS 23281; Contract grant sponsor: Ohio State Research Challenge Program.

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Received ; accepted 16 February 1997.

1995). Increases in unidirectional potassium efflux (O'Connor and Kimelberg, 1993; Sánchez-Olea et al., 1993b) are apparently balanced by equivalent increases in potassium accumulation rate (Mongin et al., 1994). In contrast, when cells are swollen in 110 mOsm conditions, a significant reduction in cellular potassium content is measured over a 30 min period (Bender and Norenberg, 1994). The osmolality dependence of changes in net content of intracellular osmolytes also is reflected in unidirectional rates of potassium and taurine efflux. Potassium efflux from cerebral astrocytes is unchanged when cells are moved from 290 mOsm media to 200 mOsm media, but it increases markedly when they are placed into a 150 mOsm solution (Sánchez-Olea et al., 1993a). Relative to this potassium efflux, loss of taurine is more sensitive to osmotic swelling. Increased taurine efflux is observed with a change in osmolality as small as 10% from an initial condition of 300 mOsm (Pasantes-Morales et al., 1990).

Taurine is the most prevalent amino acid in cultured astrocytes and has been implicated as an important osmolyte for both hypoosmotic and hyperosmotic volume regulation (Kimelberg et al., 1990b; Olson and Goldfinger, 1990; Pasantes-Morales and Schousboe, 1988; Vitarella et al., 1994). Taurine accumulation by brain cells appears to be carrier mediated and dependent upon an inwardly directed concentration gradient for sodium across the cell membrane (Holopainen et al., 1987). Mechanisms which control the efflux of this amino acid are less well understood. Loss of taurine from astrocytes in hypoosmotic conditions appears to be passive, suggesting a membrane channel or pore may mediate net efflux of this amino acid (Sánchez-Olea et al., 1991). Channels capable of conducting taurine have been observed in MDCK cells, C6 glioma cells, and human glia (Jackson and Strange, 1994; Roy, 1995; Roy and Banderali, 1994) and a large conductance anion channel sensitive to hypoosmotic medium has been characterized in cultured astrocytes (Jalonen, 1993). In C6 cells, activation of a membrane channel capable of conducting anionic taurine is closely associated with an osmotic-induced increase of organic osmolyte efflux (Jackson and Strange, 1994). In the experiments described here, we examined primary cultures of rat cerebral astrocytes exposed to various hypoosmotic conditions to determine the electrophysiological correlates of pathways which may mediate taurine, chloride, and potassium efflux. We hypothesized that the reported osmotic dependencies of unidirectional osmolyte fluxes induced by hypoosmotic swelling are reflected by similar dependencies in membrane conductance to these species.

MATERIALS AND METHODS

Primary Astrocyte Cultures

Astrocyte cultures were prepared from the cerebral cortices of 2–4 day old Sprague-Dawley rat pups (Olson and Holtzman, 1980). After dissecting the brains from the animals, meninges were carefully removed and the cerebral cortices minced and then incubated for 15 min at 37°C in Hank's balanced salt solution containing 0.15% trypsin, but without Ca^{++} or Mg^{++}. The resulting suspension was vortex mixed and filtered through sterile 80 μm nylon mesh. The filtrate was centrifuged and the supernatant discarded. Cells from the pellet were suspended in growth medium containing 20% newborn calf serum as described previously (Olson and Holtzman, 1980) and were plated at an initial density of approximately 60,000 cells/cm^2 onto nitric acid-washed 12 mm glass coverslips placed into 35 mm plastic culture dishes. Cultures were incubated at 37°C in a humidified atmosphere (90% relative humidity) containing 5% CO_2 and 95% air. After 2–3 days, and subsequently twice each week, the medium was changed to fresh growth medium that contained only 10% newborn calf serum. Cultures were confluent after 2 weeks and were examined electrophysiologically between 2 and 4 weeks in vitro. More than 90% of the cells stain positively for GFAP using an immunoperoxidase procedure (Olson and Holtzman, 1980).

Electrophysiological Studies

Coverslips containing cells were placed onto the stage of an inverted microscope and maintained at 34–36°C. In most experiments, cells were perfused with isoosmotic phosphate-buffered saline (PBS) containing (in mM) 147 NaCl, 2.7 KCl, 1.0 $CaCl_2$, 0.5 $MgCl_2$, 2.7 Na_2HPO_4, 0.5 KH_2PO_4, and 5.5 glucose (pH = 7.3, osmolality = 290 mOsm). Hypoosmotic PBS was made by reducing the concentration of NaCl. Control experiments indicated that changing the perfusing solution from isoosmotic to hypoosmotic PBS introduced a change in electrode junction potential of less than 15 mV. Reported membrane potential changes are corrected for this value at each osmolality. However, in studies designed to accurately measure cell membrane potentials during hypoosmotic swelling, changes in electrode junction potential with different perfusion solutions were minimized by using modified isoosmotic PBS solutions. These isoosmotic solutions were made by adding sucrose to hypoosmotic PBS until the measured osmolality was 290 mOsm, thus assuring equivalent ionic concentrations in each condition. With these modified solutions (isoosmotic-sucrose PBS), a variation of electrode potential of less than 2 mV was measured with each solution change. All osmolalities were verified by vapor pressure osmometry.

Electrophysiological recordings were made in the whole cell configuration. Electrodes were pulled in two steps from borosilicate glass and filled with a solution containing (in mM) 140 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 2 Mg-ATP, 10 EGTA, and 10 Hepes (pH = 7.2). Typical electrode resistances were 3–6 MΩ. In some studies, KCl was replaced with CsCl or chloride salts were replaced with their respective gluconate salts. When

determining changes in membrane conductance to anionic taurine, the electrode solution contained (in mM) 200 taurine, 1 $MgSO_4$, 10 Hepes, 1 Mg-ATP, and 1 EGTA. CsOH was used to adjust the pH to 8.2, then sufficient sucrose (approximately 50 mM) was added to elevate the osmolality to 290 mOsm. Since the apparent pK_a for the taurine amino group is 8.7 (Budavari, 1989), this resulted in a solution containing 48 mM anionic taurine. In these experiments, cells were perfused with a solution identical to the electrode solution but omitting ATP and EGTA, and hypoosmotic swelling was induced by changing to a perfusing solution without added sucrose.

We selected cells for recording which made direct cell contacts visible by phase contrast with fewer than 5 other cells. Cells were perfused with isoosmotic PBS while positioning the microelectrode on the cell to establish an electrophysiological recording. Resting membrane potentials were measured in current-clamp mode within 5 min of rupturing the cell membrane patch in the electrode tip. Data from cells were discarded if the membrane potential was not stable within this time interval. Total cell capacitance was determined in voltage clamp mode using amplifier controls (Axon Instruments 200A) to electronically compensate the current waveform in response to a voltage step from −70 mV to −63 mV. Then, using the voltage-clamp mode, astrocyte membrane currents were measured in response to 90 msec voltage steps from a holding potential of −70 mV to final values ranging between −90 mV and +30 mV. Current voltage relations for each recorded cell were determined from the average of the cell current during the last 30 msec of each voltage step. Membrane conductance for each cell was calculated by determining the slope conductance of the current-voltage relation between −100 mV and −40 mV by linear regression. These curve fits typically yielded regression coefficients (r^2) greater than 0.97, indicating that less than 3% of the variation in current over this voltage range was nonlinear. In some studies, the charging time constant of the membrane capacitance was determined by fitting the current measured in response to the voltage step from −70 mV to −90 mV to an exponential function using a nonlinear curve fitting routine. When taurine conductance was measured, the membrane potential was stepped between −80 mV and +80 mV in 20 mV steps from a holding potential of 0 mV. Membrane conductance was calculated for these cells over a range of membrane potentials from −40 mV to +40 mV.

Data Analysis

Mean conductances and membrane time constants were compared between groups by ANOVA. When data obtained at different times from the same cell were analyzed, a repeated measures ANOVA was used. These analyses were followed by Dunnett's post hoc test to determine significant differences between experimental groups.

RESULTS

With a microelectrode solution containing 140 mM KCl, the mean ± SD resting membrane potential of cells in isoosmotic PBS was −66.6 ± 15.6 mV (N = 71). Total cell capacitance ranged from 22.3 pF to 86.1 pF, while the resting cell conductance normalized to the cell capacitance was 55.6 ± 5.8 pS/pF (mean ± SEM, N = 10). Exposure of astrocytes to 190 mOsm hypoosmotic PBS caused a substantial increase in membrane conductance within several minutes while the interpolated membrane potential at zero electrode current (resting potential) depolarized by 30–40 mV (Fig. 1). To determine more accurately changes in astrocyte membrane potential during hypoosmotic swelling, the perfusion solution was changed from isoosmotic-sucrose PBS to hypoosmotic PBS (Fig. 2). In each isoosmotic-sucrose PBS, resting membrane potential was similar, with an overall mean ± SEM of −58.0 ± 3.4 mV (N = 26). Changing to 220 mOsm hypoosmotic PBS caused a mean ± SEM depolarization of 23.1 ± 5.0 mV measured 6 min later. Slightly more depolarized membrane potentials were observed after 6 min of perfusion with 190 mOsm and 145 mOsm PBS.

The increase in membrane conductance caused by exposure to hypoosmotic PBS was time dependent (Fig. 3). Cells exposed to 190 mOsm and 145 mOsm PBS showed increased conductance within 3 min. Conductance of cells perfused with 220 mOsm PBS was not significantly increased until 6 min after the start of hypoosmotic exposure. The time of maximal conductance increase occurred later with the more severe hypoosmotic treatments. This maximum change in membrane conductance was dependent upon extracellular osmolality (Fig. 4A). In contrast, assuming the electrode resistance was not altered by changing the perfusing solution, measurements of the charging time constant of the membrane indicated no change in cell capacitance to any hypoosmotic PBS treatment (Fig. 4B).

Membrane conductance also was increased in hypoosmotic PBS when the microelectrode solution contained 140 mM CsCl rather than 140 mM KCl (Fig. 5). Cells recorded using CsCl or KCl in the microelectrode solution had similar increases in relative membrane conductance when placed in 190 mOsm PBS. For each microelectrode solution, a larger increase in membrane conductance was measured at 145 mOsm. With gluconate replacing chloride in the microelectrode solution, no increase in membrane conductance was measured at 190 mOsm and the conductance increase obtained when cells were perfused with 145 mOsm PBS was smaller than that measured from cells with KCl in the microelectrode ($P < 0.05$). Finally, using a KCl microelectrode solution, similar relative conductance increases were obtained when all NaCl in the isoosmotic perfusion solution was replaced with sucrose and osmolality decreased by lowering the sucrose concentration (data not shown).

The nature of the hypoosmotically induced conductance increase of cells recorded with a KCl microelec-

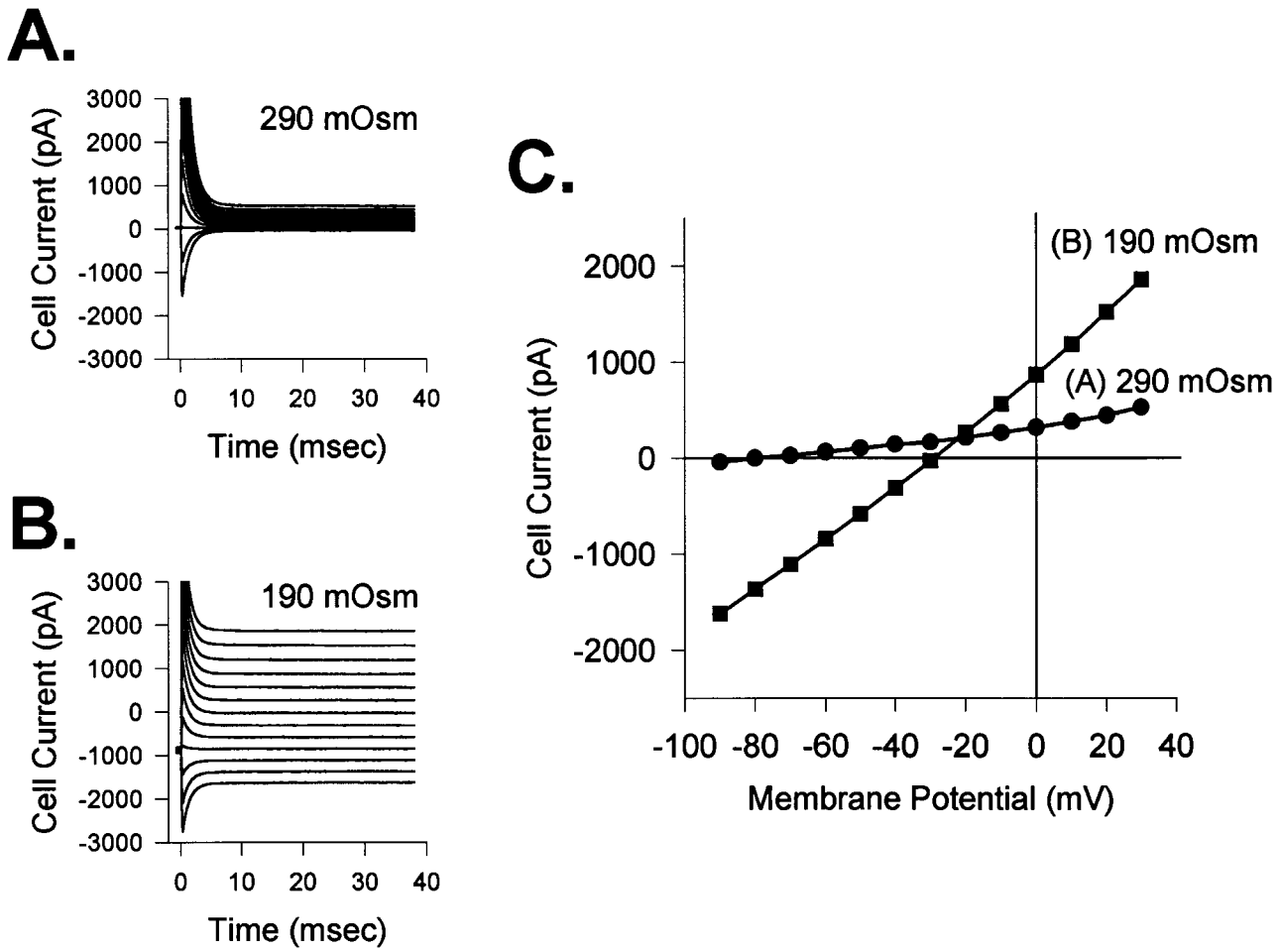

Fig. 1. Cell currents in response to membrane voltage steps of an astrocyte perfused with isoosmotic PBS (**A**) and after 6 min of perfusion with hypoosmotic PBS (**B**). Recording electrodes contained 140 mM KCl and other constituents as described in Materials and Methods. The cell membrane potential was held at −70 mV and was stepped in 10 mV increments between −90 mV and +30 mV. The charging time constant of the membrane capacitance was 1.2 msec in isoosmotic PBS and 1.1 msec in hypoosmotic PBS. (**C**) Current-voltage relationships derived from data in A and B by averaging the cell current during the last 30 msec of each voltage pulse.

trode solution was examined using pharmacologic treatments to block anion or potassium channels (Table 1). In these studies, the perfusing solution first was changed from isoosmotic PBS to hypoosmotic PBS. Three min later, the perfusing solution was changed to an identical solution containing either 200 μM niflumic acid or 5 mM $BaCl_2$. Within 9 min of exposure, niflumic acid completely inhibited the conductance increase induced by exposure to 190 mOsm PBS, while only about 60% of the conductance increase was inhibited by this drug when cells were perfused with 145 mOsm PBS. In contrast, $BaCl_2$ had no effect on the conductance increase at 190 mOsm but blocked over 80% of the increase observed at 145 mOsm.

We examined changes in astrocyte membrane conductance to taurine during exposure to hypoosmotic conditions (Fig. 6). The electrode and perfusing solutions contained anionic taurine as a major osmolyte and the major anion. A significant increase in conductance was observed upon exposure to the hypoosmotic solution. The mean ± SEM relative conductance after 3 min and 6 min of hypoosmotic exposure was 1.8 ± 0.3 and 5.1 ± 1.4, respectively ($N = 6$, $P < 0.05$). If 200 μM niflumic acid was added after 3 min of exposure to the hypoosmotic solution, 105 ± 8% (mean ± SEM) of the increase in conductance was reversed. The mean ± SEM membrane reversal potentials measured in the isoosmotic solution was −7.3 ± 3.0 mV, a value not significantly different from 0 mV. Perfusing the cells with hypoosmotic and then hypoosmotic plus niflumic acid solutions changed this membrane potential by −2.3 ± 6.7 mV and −2.0 ± 4.3 mV, respectively.

DISCUSSION

Our data demonstrate exposure of cultured astrocytes to hypoosmotic conditions induces increases in membrane conductances to both cations and anions. These conductance increases appear to be mediated by pharmacologically separate pathways which are activated by different degrees of osmotic swelling. Thus our

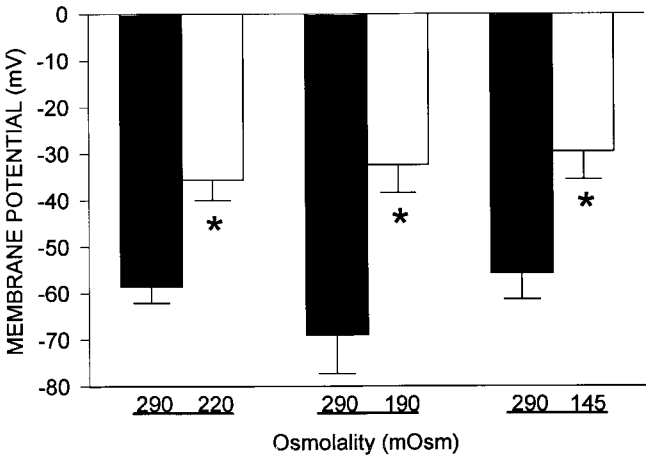

Fig. 2. Membrane potentials of astrocytes in isoosmotic-sucrose PBS (290 mOsm) and hypoosmotic PBS. Recording microelectrodes contained 140 mM KCl plus other constituents as described in Materials and Methods. Hypoosmotic PBS was made by reducing the concentration of standard PBS to achieve the desired osmolality as described in Materials and Methods. Isoosmotic-sucrose PBS was made by adding sucrose to the hypoosmotic PBS to return the osmolality to 290 mOsm. Data shown are the mean ± SEM of results from 6–12 cells. *Indicates mean values which are significantly different from the value measured in isoosmotic conditions ($P < 0.05$).

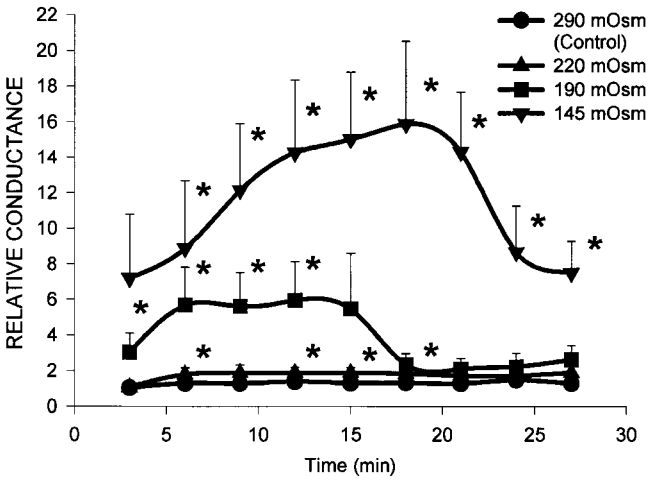

Fig. 3. Time course of astrocyte membrane conductance in response to exposure to various hypoosmotic conditions. Recording microelectrodes contained 140 mM KCl plus other constituents as described in Materials and Methods. Relative conductance is defined as the ratio of the conductance measured at each time point relative to that measured just prior to the start of hypoosmotic exposure. Each point represents the mean ± SEM of 6–11 cells. *Indicates mean values which are significantly different from unity ($P < 0.05$).

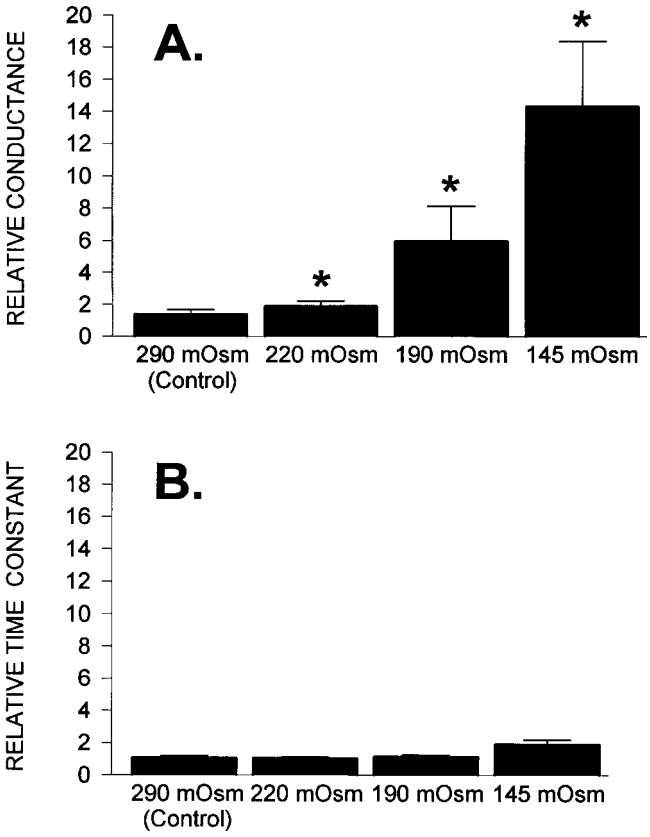

Fig. 4. Relative conductance (**A**) and relative charging time constant of the membrane capacitance (**B**) of astrocytes following exposure to hypoosmotic conditions. Recording microelectrodes contained 140 mM KCl plus other constituents as described in Materials and Methods. Relative conductance is defined as the ratio of the maximal value measured during hypoosmotic exposure relative to the value measured just prior to the start of hypoosmotic exposure. Charging time constants were calculated from the current response to a membrane voltage step as described in Materials and Methods. Relative time constant is the charging time constant measured at the time of maximal relative conductance divided by the charging time constant measured just prior to the start of hypoosmotic exposure. Each bar represents the mean ± SEM of 6–11 independent observations. *Indicates mean values which are significantly different from unity ($P < 0.05$).

data are similar to those obtained from Erlich ascites tumor cells and lymphocytes, which also show separate chloride and potassium conductance increases upon swelling mediating a volume regulatory loss of these ions (Grinstein et al., 1984; Hoffman, 1985). However, our study is the first to demonstrate differential osmotic sensitivities for these two ion pathways.

The increase in astrocyte membrane conductance occurs without an alteration in apparent membrane capacitance. Thus insertion of additional plasma membrane does not contribute to the alteration in cell conductance. Although we did not measure cell-to-cell coupling in these studies, we and others have shown extensive dye and electrical coupling between these cultured astroglial cells (Dudek et al., 1988, Dermietzel et al., 1991). Thus the constant measured capacitance during hypoosmotic exposure suggests changes in cell-to-cell coupling mediated by gap junctions do not occur upon cell swelling. Similar hypoosmotically induced increases in cell conductance which are not correlated with changes in membrane capacitance have been observed in rat hepatocytes, Jurkat lymphocytes, and myocytes (Graf et al., 1995; Ross et al., 1994; Satoh et al., 1996).

Comparisons can be made between the time course of conductance change and cell volume during swelling; however, similar comparisons during volume regulation may be problematic in this cell preparation. Vol-

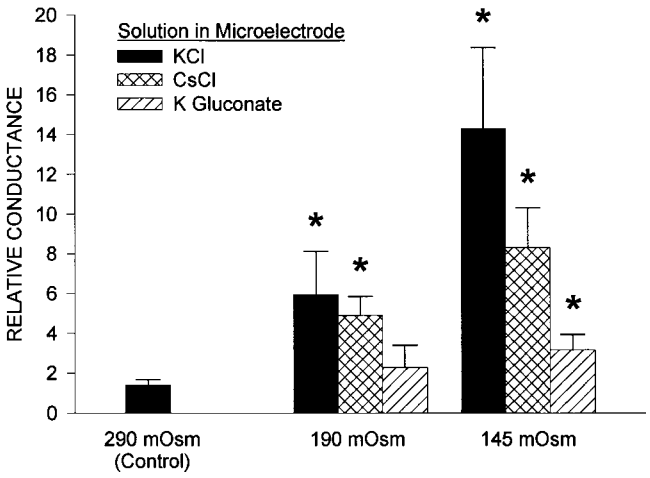

Fig. 5. Astrocyte membrane conductance in various hypoosmotic conditions. Cells were recorded with electrodes containing 140 mM KCl (solid bars), 140 mM CsCl (double-hatched bars), or 140 mM potassium-gluconate with all other chloride salts replaced by gluconate salts (single-hatched bars). Other constituents of the microelectrode solutions are described in Materials and Methods. Relative conductance is defined as described for Figure 4. Variability of the control conductance was calculated from cells maintained over a similar period of time in 290 mOsm PBS. Each bar represents the mean ± SEM of 6–9 independent observations. *Indicates mean values which are significantly different from unity ($P < 0.05$).

TABLE 1. Osmotic dependence of niflumic acid and $BaCl_2$ inhibition of astrocyte membrane conductance increases induced by hypoosmotic exposure[a]

	Percent drug inhibition	
Drug additive	190 mOsm	145 mOsm
5 mM $BaCl_2$	7.2 ± 6.8% (3)	82.4 ± 15.0%[b] (10)
200 μM niflumic acid	114.1 ± 21.7%[b] (3)	60.6 ± 11.4%[b] (6)

[a]Values are the mean ± SEM percent inhibition of cell conductance induced by hypoosmotic exposure for the number of independent observations shown in parentheses.
[b]Indicates mean values which are significantly different from zero ($P < 0.05$).

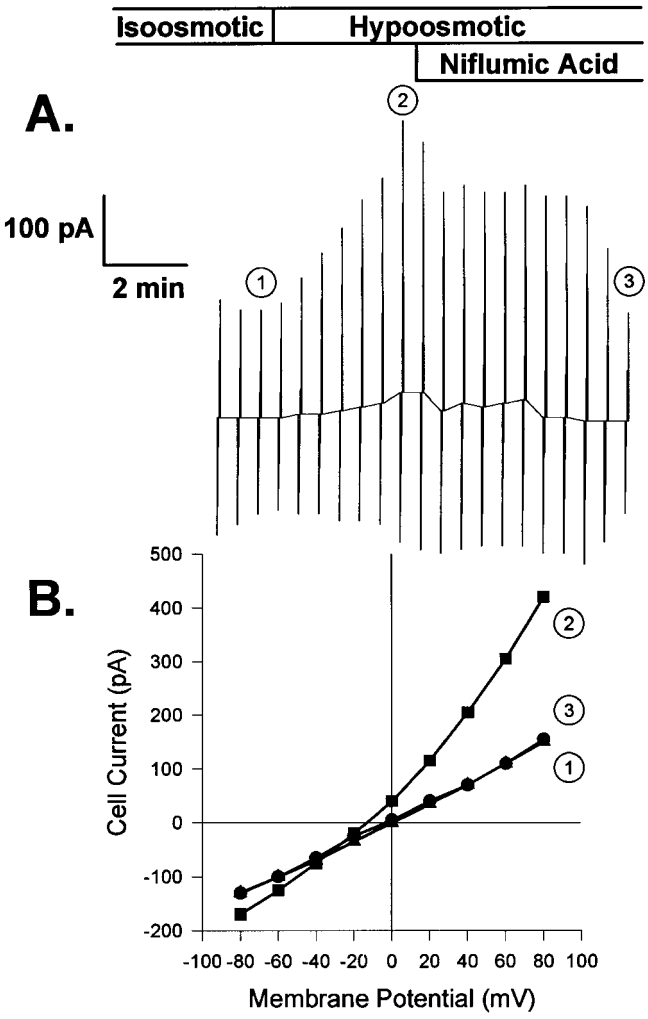

Fig. 6. Membrane currents recorded from a cell in whole cell patch configuration with 200 mM taurine and 50 mM sucrose (pH 8.2) in the microelectrode and perfusion solutions. Other solution constituents are described in Materials and Methods. At the indicated times the perfusion solution was made hypoosmotic by changing to a solution without added sucrose. Niflumic acid (200 μM) was added to the hypoosmotic solution 3 min later. **A:** Current responses from a holding potential of 0 mV to membrane voltages ranging from −80 to +80 mV in 20 mV steps. Holding currents measured between each period of data acquisition are connected by straight lines. **B:** Current voltage relationships derived from data in A in isoosmotic conditions (1, circles), hypoosmotic conditions (2, squares), and hypoosmotic conditions after 6 min of exposure to 200 μM niflumic acid (3, triangles).

ume measurements of astrocytes after acute exposure to hypoosmotic conditions typically demonstrate maximal swelling within the first 1–3 min (Bender and Norenberg, 1994; Kimelberg and Frangakis, 1995; Olson et al., 1996). Subsequently a regulatory volume decrease occurs over 15–20 min as intracellular osmolytes are lost from the cells. The relatively slower time course of conductance increase observed in these studies compared with the expected initial rate of swelling suggests that cell volume does not directly affect the ion pathways as would be expected if membrane stretch channels similar to those identified in these (Bowman et al., 1992) and other cells (Christensen, 1987) were responsible for the conductance change. Because the cell interior is continually dialyzed by the isoosmotic microelectrode solution, cell volume decrease may not occur in the recorded cells. Rather, after an initial period of rapid swelling, continued cell swelling is expected to occur until the rate of osmolyte entry via dialysis into the cell from the microelectrode is equal to the rate of osmolyte loss through the cell membrane. Initially, equilibration of the hypoosmotic cell interior with the electrode solution is expected to take several minutes (Pusch and Neher, 1988). However, the rate of ion diffusion from the electrode will decease with time as more of the solution at the tip of the microelectrode becomes diluted by diffusion through the cell to the hypoosmotic perfusion solution. Thus the time course of conductance increase observed in these studies may reflect activation of ion channels mediated by a biochemical pathway initiated by the swelling event, as well as the effects of progressive cell swelling occurring over several minutes. The fall in membrane conduc-

tance after 6–20 min of hypoosmotic exposure may be due to deactivation of the channels or as an indirect effect of the regulatory cell shrinkage once the microelectrode solution at the tip of the electrode becomes diluted by continual diffusion into the cell interior. Further studies using a perforated patch configuration to retard equilibration of the cell interior with the microelectrode solution may provide further insight into the mechanism(s) responsible for the time course of cell conductance changes.

The rate of taurine efflux from astrocytes shows an osmolality-dependent rise between 300 mOsm and 150 mOsm (Pasantes-Morales et al., 1990). Previous studies also have suggested taurine loss is highly correlated with volume regulation in 100 mOsm medium (Vitarella et al., 1994). In contrast, astrocyte potassium contents are not changed after exposure to 175 mOsm conditions (Olson and Kimelberg, 1995), while significant potassium losses occur during volume regulation in 110 mOsm medium (Bender and Norenberg, 1994). In parallel with these studies of cellular efflux and content, the present studies demonstrated niflumic acid-sensitive conductance pathways permeable to chloride and taurine activated by 220–190 mOsm conditions. Potassium conductance increase was observed at 145 mOsm similar to the net loss of potassium observed at these larger osmotic insults. Although inhibition of membrane conductance at 145 mOsm was obtained with both $BaCl_2$ and niflumic acid, the arithmetic sum of the inhibitory effects was greater than 100%. This may indicate significant variability between cells in the relative contribution of anion and cation conductance at 145 mOsm or it may indicate an effect of niflumic acid on potassium conductance under these extreme hypoosmotic conditions.

Membrane depolarization observed at each hypoosmotic exposure is consistent with activation of chloride conductance. Using the Goldman-Hodgkin-Katz voltage equation for a membrane at 37°C permeable to sodium, potassium, and chloride (Hille, 1992), and assuming constant potassium permeability (P_K), constant sodium permeability (P_{Na}), and $P_K/P_{Na} = 30$ (Kimelberg et al., 1979), the membrane potential change observed with perfusion of 220 mOsm PBS is predicted with a 5.4-fold increase in chloride permeability (P_{Cl}). Further increases in P_{Cl} at 190 mOsm and 145 mOsm are predicted with these same assumptions. Slightly smaller changes in P_{Cl} are calculated if P_{Na} is neglected. Increased sodium and calcium influx during hypoosmotic swelling also have been observed in these glial cells (Bender and Norenberg, 1994; Olson and Evers, 1992). Thus some of the membrane depolarization in hypoosmotic conditions may be due to activation of pathways permeable to these cations. Since similar electrophysiological changes were observed with all extracellular NaCl replaced by sucrose, sodium is not likely to be a major charge carrier under the conditions of these experiments. However, we cannot rule out a contribution by calcium.

Currents activated by brief (30 sec) hypoosmotic exposures of approximately 190 mOsm also have been reported (Kimelberg et al., 1990a). In these studies, a transient conductance increase occurred several minutes after the cell was returned to isoosmotic conditions, suggesting that increased cell volume did not directly activate membrane channels. In contrast to the present study, no anion current was detected. Thus activation of a chloride conductance as we observed may require a more prolonged period of cell swelling. Other studies have implicated calcium or calcium/calmodulin-dependent mechanisms, leading to increased potassium conductance in swollen astrocytes (Bender and Norenberg, 1994; Olson et al., 1990). This osmolyte flux may be mediated by the calcium-activated potassium channels described in these cells (Quandt and MacVicar, 1986).

Ion channels which may mediate the cell conductance changes reported here have been identified previously in these cells. Additionally, in glia and in other cell types, anion channels have been implicated in osmotically dependent loss of taurine and other amino acids (Jackson and Strange, 1994; Roy and Banderali, 1994). Jalonen described a high conductance (200–300 pS) anion channel activated by hypoosmotic exposure of the cell or by a hypoosmotic gradient imposed on an isolated membrane patch (Jalonen, 1993). This channel demonstrated a voltage-dependent open probability which was maximal (approximately 0.9–1.0) at 0 mV with symmetrical bath solutions on the membrane patch. Our studies did not determine cell conductance at membrane voltages in the range of maximal activation of this channel. Rather, we choose a range of membrane potentials likely to occur in astrocytes in isoosmotic and hypoosmotic conditions (Kimelberg and O'Connor, 1988; Walz et al., 1984). At these membrane voltages, the osmolality-sensitive anion channel has an open probability of approximately 0.25 (Jalonen, 1993).

In this study we demonstrated osmotically sensitive chloride and taurine conductances which are inhibited by niflumic acid, known to inhibit volume regulation and reduce hypoosmotically induced taurine efflux in vitro (Law, 1994; Sánchez-Olea, 1993b). The high pH necessary to ionize a significant proportion of the intracellular and extracellular taurine is not expected to alter mechanisms which lead to taurine loss since osmotically induced efflux of taurine is insensitive to changes in extracellular pH between about 6.5 and 8.5 (Pasantes-Morales et al., 1990). While these limited observations do not demonstrate that taurine and chloride are transported out of the cells by the same conductance pathway, the similarity of these data to observations in C6 and human glioma cells and MDCK epithelial cells, where this association has been more firmly established, and the data from others showing inhibition of a hypoosmotically induced anion channel by agents known to block astrocyte volume regulation and taurine transport, make this a tenable hypothesis which may be more fully evaluated in future studies.

ACKNOWLEDGMENTS

The authors are grateful for the assistance of Julie Evers and Rhonda Barton, who prepared and maintained the astrocyte cell cultures. Jay Dean and Richard White provided helpful comments during the design of the experiments and critical review of this manuscript. This research was supported by Kettering Medical Center and by grants to J.E.O. from the National Institutes of Health (NS 23281) and the Ohio State Research Challenge Program.

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