The Journal of Neuroscience, May 15, 1998, *18*(10):3554–3562

Effects of Glucose Deprivation, Chemical Hypoxia, and Simulated Ischemia on Na^+ Homeostasis in Rat Spinal Cord Astrocytes

Christine R. Rose,[1,2] **Stephen G. Waxman,**[1,2] **and Bruce R. Ransom**[3]

[1]*Department of Neurology, Yale University School of Medicine, New Haven, Connecticut 06520,* [2]*Neuroscience Research Center, VA Hospital, West Haven, Connecticut 06516, and* [3]*Department of Neurology, University of Washington School of Medicine, Seattle, Washington 98195-6465*

A steep inwardly directed Na^+ gradient is essential for glial functions such as glutamate reuptake and regulation of intracellular ion concentrations. We investigated the effects of glucose deprivation, chemical hypoxia, and simulated ischemia on intracellular Na^+ concentration ($[Na^+]_i$) in cultured spinal cord astrocytes using fluorescence ratio imaging with sodium-binding benzofuran isophthalate (SBFI) AM. Glucose removal or chemical hypoxia (induced by 10 mM NaN_3) for 60 min increased $[Na^+]_i$ from a baseline of 8.3 to 11 mM. Combined glycolytic and respiratory blockage by NaN_3 and 0 glucose saline caused $[Na^+]_i$ to increase by 20 mM, similar to the $[Na^+]_i$ increases elicited by blocking the Na^+/K^+-ATPase with ouabain. Recovery from large $[Na^+]_i$ increases (>15 mM) induced by the glutamatergic agonist kainate was attenuated during glucose deprivation or NaN_3 application and was blocked in NaN_3 and 0 glucose. To mimic *in vivo* ischemia, we exposed astrocytes to NaN_3 and 0 glucose saline containing L-lactate and glutamate with increased $[K^+]$ and decreased $[Na^+]$, $[Ca^{2+}]$, and pH. This induced an $[Na^+]_i$ decrease followed by an $[Na^+]_i$ rise and a further $[Na^+]_i$ increase after reperfusion with standard saline. Similar multiphasic $[Na^+]_i$ changes were observed after NaN_3 and 0 glucose saline with only reduced $[Na^+]_e$. Our results suggest that the ability to maintain a low $[Na^+]_i$ enables spinal cord astrocytes to continue uptake of K^+ and/or glutamate at the onset of energy failure. With prolonged energy failure, however, astrocytic $[Na^+]_i$ rises; with loss of their steep transmembrane Na^+ gradient, astrocytes may aggravate metabolic insults by carrier reversal and release of acid, K^+, and/or glutamate into the extracellular space.

Key words: glia; hypoglycemia; anoxia; sodium-binding benzofuran isophthalate; Na^+/K^+-ATPase; kainate; excitotoxicity

The nervous system has one of the highest metabolic rates in the body and, because it stores very little fuel, depends on a continuous exogenous supply of metabolic substrate and O_2. Approximately 50% of cellular energy is needed for ion transport and the maintenance of ion gradients, mainly by the Na^+/K^+-ATPase (Hansen, 1985; Ames et al., 1992; Erecinska and Silver, 1994). Na^+/K^+-ATPase extrudes Na^+ ions in exchange for extracellular K^+ and generates an inwardly directed gradient for Na^+ ions, which in turn energizes other secondary ion transporters (e.g., Na^+–Ca^{2+} exchange). Pathological conditions that diminish or arrest energy production such as hypoglycemia or anoxia/ischemia, therefore, lead to rapid breakdown of cellular ion gradients (Hansen, 1985; Erecinska and Silver, 1994).

Breakdown of the Na^+ gradient after inhibition of Na^+/K^+-ATPase is probably one of the key elements in promoting cellular damage during energy failure (Lees, 1991; Lucas et al., 1997), and blocking Na^+ influx can protect neurons from anoxic injury (Stys et al., 1992; Agrawal and Fehlings, 1996; Imaizumi et al., 1997). Astrocytes are much less susceptible to hypoglycemia or ischemia than neurons (Auer and Siesjö, 1988; Ransom and Fern, 1996), in part because they possess larger glycogen stores (Hamprecht and Dringen, 1995) and are less subject to glutamatergic excitotoxicity [Choi and Rothman (1990); see, however, David et al. (1996)].

In addition, astrocytes can increase survival of neurons during metabolic insults (Vibulsreth et al., 1987; Swanson and Choi, 1993). This protection may be attributable to the release of lactate by astrocytes (Walz and Mukerji, 1988; Tsacopoulos and Magistretti, 1996) or to their ability to regulate extracellular glutamate (Rosenberg, 1991) or extracellular ion concentrations (Kraig et al., 1995). Other studies suggest, however, that astrocytes may aggravate ischemic damage because of extensive swelling and/or release of glutamate (Siesjö, 1985; Billups and Attwell, 1996), both of which probably occur secondary to increases in intracellular Na^+ concentration ($[Na^+]_i$). To elucidate the mechanisms of hypoglycemic or ischemic damage, therefore, one must understand precisely ion movements not only in neurons but also in glial cells in response to these metabolic insults. Culture systems are well suited for investigating altered ion regulation, because, in contrast to the situation *in vivo*, the extracellular conditions can be manipulated and controlled rigorously.

In the present study, we measured changes in $[Na^+]_i$ and the capacity for $[Na^+]_i$ regulation in cultured spinal cord astrocytes during glucose deprivation, chemical hypoxia, and simulated ischemia. Similar metabolic insults can affect the *in vivo* spinal cord (e.g., after trauma) and play a significant role in the establishment of axonal injury (Tator and Fehlings, 1991). Our results indicate that spinal cord astrocytes are able to maintain a steep inwardly directed Na^+ gradient for tens of minutes during energy disruption and could, therefore, play a protective role during this

Received Dec. 13, 1997; revised March 2, 1998; accepted March 2, 1998.

This work was supported in part by National Institutes of Health Grant NS-15589 to B.R.R., National Multiple Sclerosis Society Grant RG 1912, and a grant from the Medical Research Service, Veterans Administration, to S.G.W. C.R.R. was supported in part by a fellowship from the Eastern Paralyzed Veterans Association. We thank Dr. Ray Swanson for helpful comments on an earlier version of this manuscript.

Correspondence should be addressed to Dr. Christine R. Rose, I. Physiologisches Institut, Universität des Saarlandes, D-66421 Homburg/Saar, Germany.

 0270-6474/98/183554-09$05.00/0

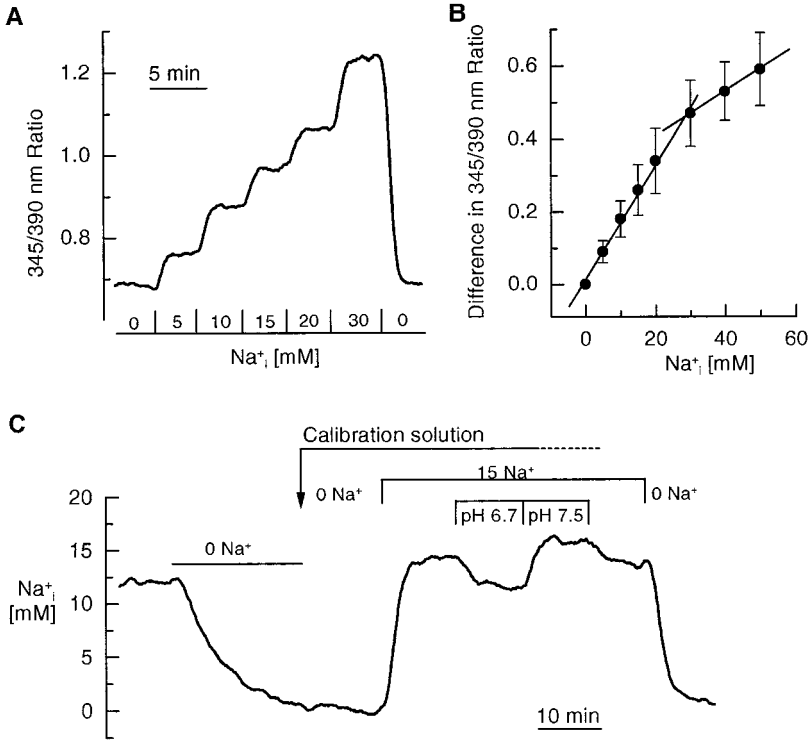

Figure 1. Intracellular calibration of SBFI fluorescence in spinal cord astrocytes. *A*, When astrocytes were perfused with calibration solutions containing the ionophores gramicidin and monensin, stepwise changes in bath Na^+ concentrations resulted in stepwise changes in the 345/390 nm fluorescence ratio of intracellular SBFI. *B*, During perfusion with calibration solution, the SBFI ratio (345/390 nm) changed monotonically with changes in $[Na^+]_i$. Data points are mean values ± SD obtained from at least 40 individual cells. *C*, In 0 Na^+ saline, $[Na^+]_i$ drops rapidly to near zero. Changing at this point to a calibration solution with 0 Na^+ did not change the fluorescence value. With constant $[Na^+]_i$ (15 mM), changing pH_i from the standard value of 7.1 to 6.7 and then to 7.5 resulted in ratio changes mimicking changes in $[Na^+]_i$, with acidifications leading to an apparent decrease and alkalinizations leading to an apparent increase in $[Na^+]_i$.

period. Because of diminished capacity for Na^+ extrusion during prolonged energy failure, however, the astrocytic Na^+ gradient is gradually reduced, promoting reversal of astrocytic carriers and cellular damage.

MATERIALS AND METHODS

Cell culture. Cell cultures of spinal cord astrocytes were prepared from neonatal rats (Sprague Dawley rats; postnatal day 0) as described previously (Black et al., 1993). Briefly, the pups were anesthetized by CO_2 and decapitated, and their spinal cords were removed. The tissue was digested with papain (20 U/ml for 30 min; Worthington, Freehold, NJ) and triturated. The dissociated cells were plated onto poly-L-ornithine- and laminin-coated (Sigma, St. Louis, MO) coverslips in complete medium (Earle's minimum essential medium; JRH Biologicals, Lenexa, KA) containing 10% fetal bovine serum (HyClone, Logan, UT), 6 mM glucose, and penicillin and streptomycin at 120 U/ml and 120 μg/ml, respectively (Sigma). Cells were grown in an incubator at 37°C and 95 %O_2/5% CO_2 and were fed every 3–4 d. Cells between 14 and 21 d in culture were used for experiments. At this time *in vitro*, cultures were confluent, and nonstellate astrocytes [cf. Sontheimer et al. (1992), their Fig. 1] represented >95% of the cells on a given coverslip, as revealed by immunostaining for glial fibrillary acidic protein.

Solutions. During experiments, cells were perfused with standard saline containing (in mM): 140.7 NaCl, 3 KCl, 1.2 $MgSO_4$, 1 $CaCl_2$, 2 NaH_2PO_4, 20 HEPES, and 10 glucose, titrated to a pH of 7.4 with NaOH. In salines with altered ionic composition (e.g., increased $[K^+]$), the NaCl concentration was reciprocally changed to maintain constant osmolarity. The sensitivity of SBFI to Na^+ was calibrated after each experiment with salines that contained (in mM): 150 $[K^+ + Na^+]$, 30 Cl^-, 120 gluconic acid, and 10 HEPES, titrated to pH 6.9 with KOH (cf. Rose and Ransom, 1996a). In addition, these calibration solutions contained 3 μM gramicidin and 10 μM monensin for equilibration of extra- and intracellular $[Na^+]$.

Drugs and chemicals were purchased from Sigma and added to the saline shortly before use. Gramicidin, monensin, and ouabain were prepared as 1 M stock solutions in dimethylsulfoxide (DMSO; Sigma) and stored in the freezer. Acetoxymethyl ester of sodium-binding benzofuran isophthalate (SBFI AM) was obtained from Teflabs.

Measurement of $[Na^+]_i$. $[Na^+]_i$ was measured using fluorescence ratio imaging with SBFI AM as described previously (Rose and Ransom, 1996a; Rose et al., 1997). In summary, cells were loaded with SBFI AM (20 μM; 90 min) in standard saline containing 0.1% pluronic (BASF/Knoll, Ludwigshafen, Germany) at room temperature (20–22°C). After dye-loading, the coverslips were transferred to an experimental chamber (volume, ~500 μl) and perfused with saline warmed to 37°C at a flow rate of ~1 ml/min. The chamber was mounted on the stage of a Nikon-Diaphot TMD inverted microscope equipped with an oil-immersion objective (Nikon Fluor 40/1.30). The cells were excited every 10 sec at 345 and 390 nm, and emission fluorescence of single cells above 510 nm was collected, averaging four video frames. The collection was performed by a GenIISys image intensifier system connected to a videocamera (MTI CCD72; Dage-MTI, Michigan City, IN). Fluorescence was quantified using an image acquisition program from Georgia Instruments (Roswell, GA).

Absolute $[Na^+]_i$ was determined for each individual cell by a two- or three-point calibration performed after each experiment (Rose and Ransom, 1996a; Rose et al., 1997). For calibration of the fluorescence of SBFI, the astrocytes were perfused with calibration solutions containing known Na^+ concentrations (0 and 30 mM or 0, 30, and 50 mM $[Na^+]$) and gramicidin and monensin (see above) for equilibration of extra- and intracellular ion concentrations. The 345/390 nm fluorescence ratio of intracellular SBFI changed monotonically with changes in $[Na^+]_i$; stepwise changes in Na^+ concentrations resulted in stepwise changes in the SBFI ratio (Fig. 1*A*). The slope of the ratio change was 0.16 per 10 mM Na^+ from 0 to 30 mM $[Na^+]_i$ ($n = 47$) and 0.07 per 10 mM Na^+ for 30–60 mM $[Na^+]_i$ ($n = 40$; Fig. 1*B*).

As reported earlier (Rose and Ransom, 1996a), the K^+ sensitivity of intracellular SBFI was negligible. Reduction of $[K^+]_i$ by 40 mM resulted in an apparent decrease in $[Na^+]_i$ of <2 mM (data not shown). Intracellular SBFI fluorescence ratio did, however, react slightly to changes in pH_i. Switching pH_i from the standard value of 7.1 to 6.7 and then to 7.5 resulted in ratio changes mimicking $[Na^+]_i$ changes of ~3 mM ($n = 50$; Fig. 1*C*). Acidifications mimicked a decrease, and alkalinizations mimicked an apparent increase in $[Na^+]_i$. This weak pH sensitivity of SBFI might lead to an underestimation of $[Na^+]_i$ increases by a few millimolar in the present study during manipulations that can be accompanied by large acidifications such as simulated ischemia (Kraig and Chesler, 1990). It is unlikely, however, that the pH_i of astrocytes would change so markedly under our *in vitro* conditions because it is strongly influenced by pH_o which was held constant in these experiments (Pappas et al., 1996)

Experiments were repeated on at least four different coverslips. Data

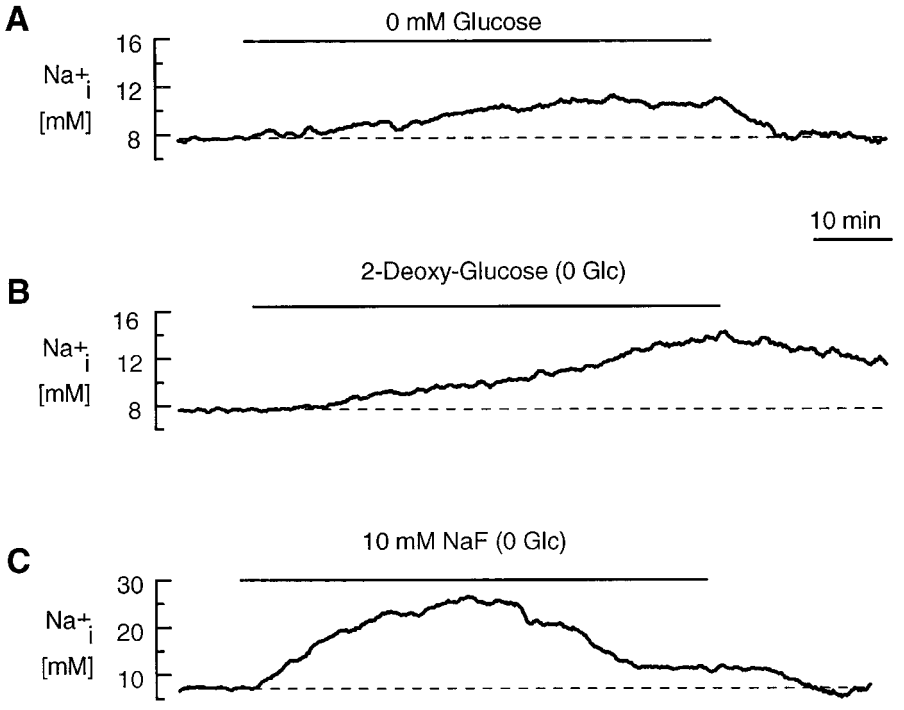

Figure 2. Changes in baseline $[Na^+]_i$ caused by reduction of glycolysis in spinal cord astrocytes. Removal of glucose (*A*) or replacement of glucose by the nonmetabolizable analog 2-deoxyglucose (*B*) caused a slow increase in astrocytic $[Na^+]_i$ by several millimolar. In contrast to the effects of glucose removal on $[Na^+]_i$, which were easily reversible, the $[Na^+]_i$ increase induced by 2-deoxyglucose was not fully reversed after a 20 min perfusion with standard saline. *C*, Inhibition of glycolysis and pyruvate dehydrogenase by 10 mM NaF with concomitant removal of glucose caused a transient $[Na^+]_i$ increase that recovered toward baseline $[Na^+]_i$ with NaF still present. Baseline $[Na^+]_i$ is indicated by the *dashed lines*. All manipulations were performed for 1 hr as indicated by the *horizontal bars* above the records. *Glc*, Glucose.

are presented as mean ± SD and were statistically analyzed using a Student's *t* test (significance level, $p < 0.001$ unless stated otherwise).

RESULTS

In agreement with a previous report [Rose et al. (1997); see also Rose and Ransom (1996a)], baseline $[Na^+]_i$ was 8.3 ± 4.0 mM ($n = 872$) in cultured spinal cord astrocytes in standard, HEPES-buffered saline, resulting in a calculated reversal potential for Na^+ (E_{Na}) of +78 mV. $[Na^+]_i$ was very stable (± 1 mM over periods of 100 min) when no other experimental manipulation was performed. To investigate the importance of cellular energy production for maintenance of this low baseline $[Na^+]_i$, we monitored $[Na^+]_i$ before, during, and after metabolic inhibition by removal of glucose, inhibition of glycolysis, and/or chemical hypoxia for periods of 1 hr. We also tested the capacity of astrocytes to recover from imposed $[Na^+]_i$ loads under these conditions. In addition, the $[Na^+]_i$ changes induced by simulated ischemia were investigated.

Effects of 0 glucose and/or glycolytic inhibition on baseline $[Na^+]_i$

The energy metabolism of astrocytes was reduced by (1) removal of glucose from the saline, (2) replacement of glucose with 10 mM 2-deoxyglucose (2-DG), or (3) addition of the glycolytic inhibitor NaF with concomitant glucose removal. Glucose deprivation alone does not result in complete inhibition of glycolysis, because glucose-6-phosphate can be provided from cellular glycogen stores and by gluconeogenesis (Erecinska and Silver, 1994; Hamprecht and Dringen, 1995). 2-DG, on the other hand, is phosphorylated to 2-DG-6-phosphate but is not metabolized further and, therefore, accumulates intracellularly, progressively blocking glycolysis and reducing glycogenolysis by inhibition of glycogen phosphorylase (Sokoloff et al., 1977; Hamprecht and Dringen, 1995). NaF, finally, not only inhibits glycolysis but also the pyruvate dehydrogenase complex (Erecinska and Silver, 1994), blocking flux of pyruvate into the citric acid cycle and reducing substrate recruitment from glycogenolysis and gluconeogenesis for respiratory oxidation. Of course, none of these approaches inhibits glycolysis in isolation. They all reduce pyruvate, the primary substrate for oxidative metabolism, at the same time.

Both glucose removal and replacement of glucose by 2-DG for a 60 min period led to a slow increase in $[Na^+]_i$ in spinal cord astrocytes that was visible 5–10 min after the onset of the manipulation (Fig. 2*A*,*B*). Glucose removal significantly increased $[Na^+]_i$ to 11.6 ± 6.6 mM ($n = 143$; see Fig. 4); this increase was completely reversible within 5–10 min after reperfusion with standard saline (Fig. 2*A*). Replacement of glucose with 2-DG caused a statistically significant higher $[Na^+]_i$ increase to 14.9 ± 6.4 mM ($n = 48$; see Fig. 4), which was only partly reversed after a 20 min wash with standard saline (Fig. 2*B*).

Concomitant removal of glucose and addition of 10 mM NaF led to a transient increase in $[Na^+]_i$ to 21.3 ± 13.2 mM ($n = 42$), which peaked within 30 min and was followed by a decline in $[Na^+]_i$. At the end of the 1 hr exposure to the glucose-free NaF-containing saline, $[Na^+]_i$ had reached 15.2 ± 8.2 mM, a value significantly higher than the $[Na^+]_i$ in standard saline or during glucose deprivation (see Figs. 2*C*, 4). The NaF-induced $[Na^+]_i$ changes were more rapid in onset than were those with glucose-free saline or application of 2-DG and reversed within 10–15 min after switching back to standard saline (Fig. 2*C*).

$[Na^+]_i$ changes induced by chemical hypoxia and ouabain

To investigate the role of aerobic metabolism in maintaining low baseline $[Na^+]_i$ in cultured spinal cord astrocytes, we inhibited mitochondrial respiration by the addition of 10 mM sodium azide (NaN_3) in the presence or absence of glucose in the saline (conditions corresponding to normal or reduced glycolytic capacity, respectively). NaN_3 blocks the oxygen-requiring steps in energy metabolism by inhibition of cytochrome oxidase and, therefore, induces a "chemical hypoxia" (Swanson, 1992).

Addition of NaN_3 for 1 hr, with glucose present, caused a small but significant increase in astrocytic $[Na^+]_i$ to 10.6 ± 4.1 mM

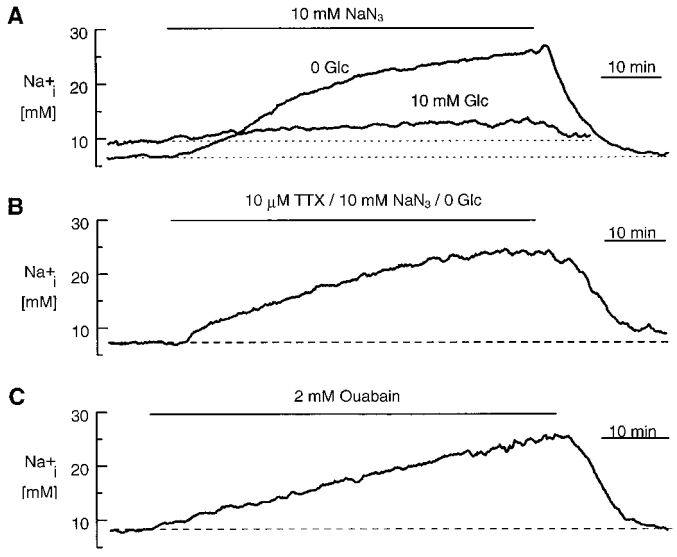

Figure 3. $[Na^+]_i$ changes induced by glucose deprivation and chemical hypoxia and by ouabain. *A*, Induction of chemical hypoxia by NaN_3 in the presence of glucose caused a small increase in $[Na^+]_i$. NaN_3 coupled with 0 glucose (0 *Glc*) caused a reversible increase in $[Na^+]_i$ by ~20 mM. *B*, The amplitude and time course of this $[Na^+]_i$ increase were not altered by blocking voltage-gated Na^+ channels with 10 μM tetrodotoxin (*TTX*). *C*, Inhibition of Na^+/K^+-ATPase activity by 2 mM ouabain (in the presence of 10 mM glucose) induced an $[Na^+]_i$ increase similar to the one observed with NaN_3 and 0 glucose. All manipulations were performed for 1 hr as indicated by the *horizontal bars*.

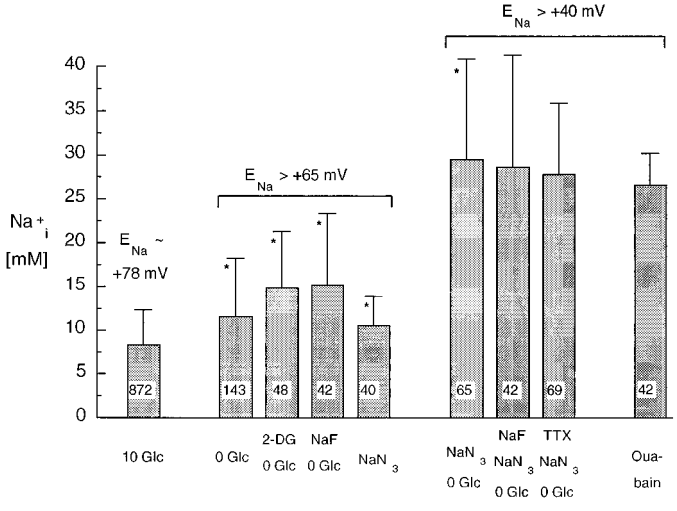

Figure 4. Histogram summarizing the effects of metabolic inhibition on baseline $[Na^+]_i$. The mean values (± SD) of $[Na^+]_i$ measured 60 min after perfusion with the different salines and the number of cells investigated (*bold numbers*) are shown. Inhibition of either glycolysis or oxidative phosphorylation caused $[Na^+]_i$ to increase to 15 mM maximally and reduced the calculated Na^+-reversal potential (E_{Na}) to +65 mV maximally (*columns 2–5*). Much larger $[Na^+]_i$ changes were induced by inhibition of both glycolysis and oxidative phosphorylation (*columns 6–8*) as well as by inhibition of Na^+/K^+-ATPase by ouabain (*column 9*). These manipulations increased $[Na^+]_i$ to 30 mM maximally and resulted in a reduction of the calculated E_{Na} to +44 mV. *Asterisks* indicate significant differences between mean and control values ($p \leq 0.001$).

($n = 40$; Figs. 3*A*, 4). $[Na^+]_i$ rose to 27.5 ± 11.3 mM when NaN_3 was applied in a glucose-free saline (NaN_3 and 0 glucose; $n = 65$; Figs. 3*A*, 4), a result significantly different than that seen with NaN_3 in the presence of glucose. This $[Na^+]_i$ increase started 3–5 min after the onset of the perfusion with NaN_3 and 0 glucose saline. The cells recovered within 10–15 min from this Na^+ load (Fig. 3*A*).

Voltage-gated Na^+ channels have been proposed to mediate significant Na^+ influx into neurons under conditions of metabolic failure (Stys et al., 1992). Because cultured spinal cord astrocytes are characterized by a high density of voltage-gated Na^+ channels (Sontheimer et al., 1992), we investigated whether the $[Na^+]_i$ increase during chemical hypoxia was attributable to channel-mediated Na^+ influx. Blocking Na^+ channels with 10 μM tetrodotoxin, however, did not visibly influence the slope or the amplitude of the $[Na^+]_i$ increase induced by NaN_3 and 0 glucose ($n = 69$; Figs. 3*B*, 4).

Metabolic inhibition lowers intracellular ATP concentration, which leads to reduced Na^+/K^+-ATPase activity (Hansen, 1985; Lees, 1991). To test the effect of Na^+/K^+-ATPase inhibition on $[Na^+]_i$ in cultured spinal cord astrocytes, we applied 2 mM ouabain for 1 hr. A concentration of 1 mM ouabain was reported to inhibit Na^+/K^+-ATPase activity in astrocyte cultures completely (Walz and Hertz, 1982).

Ouabain increased $[Na^+]_i$ within 1–2 min after its application. The ouabain-induced $[Na^+]_i$ increase ($n = 42$) was similar to that induced by metabolic inhibition using NaN_3 and 0 glucose (Figs. 3*C*, 4), indicating that metabolic inhibition probably affected $[Na^+]_i$ by leading to failure of Na^+/K^+-ATPase activity. This was also supported by the observation that adding another glycolytic blocker, NaF, to the combined glycolytic and respiratory inhibition by NaN_3 and 0 glucose did not augment the $[Na^+]_i$ increase seen with NaN_3 and 0 glucose alone ($n = 42$; Fig. 4).

Figure 4 summarizes the effects of metabolic inhibition on baseline $[Na^+]_i$ in spinal cord astrocytes by showing the mean values of $[Na^+]_i$ 60 min after the onset of the manipulation. Inhibition of glycolysis by 2-DG or NaF added to glucose-free saline increased $[Na^+]_i$ to ~15 mM. Inhibition of oxidative phosphorylation alone by NaN_3 increased $[Na^+]_i$ by only 3 mM to ~11 mM, and this increase was significantly less than that seen with glycolytic inhibition. Removal of glucose also produced only a small increase in $[Na^+]_i$, presumably because astrocytes could breakdown glycogen for fuel under these conditions.

A greater reduction in calculated E_{Na} (to +44 mV maximally) was observed with combined inhibition of glycolysis and oxidative phosphorylation for 60 min. A similar value for E_{Na} was calculated with inhibition of Na^+/K^+-ATPase activity by ouabain (Fig. 4). Even assuming that the astrocytes were depolarized under these conditions (Walz et al., 1993), the positive E_{Na} still indicates a large inwardly directed Na^+ gradient.

Recovery from imposed $[Na^+]_i$ loads during metabolic inhibition

Because inhibition of glycolysis and/or oxidative phosphorylation caused relatively small reductions in transmembrane Na^+ gradient after 60 min, we tested the ability of the astrocytes to recover from additional Na^+ loads under these conditions. $[Na^+]_i$ was increased by lowering extracellular $[K^+]$ ($[K^+]_e$), which inhibits Na^+/K^+-ATPase (cf. Rose and Ransom, 1996a), or by application of the glutamatergic agonist kainate (Kimelberg et al., 1989; Rose et al., 1997).

Removal of $[K^+]_e$ for 10 min reversibly increased $[Na^+]_i$ by 3.2 ± 2.5 mM ($n = 55$; Fig. 5). Neither the average amplitude nor the recovery from this $[Na^+]_i$ increase was significantly altered in glucose-free saline ($[Na^+]_i$ increased by 4.0 ± 1.5 mM; $n = 47$; Fig. 5), indicating that glucose removal and the subsequent in-

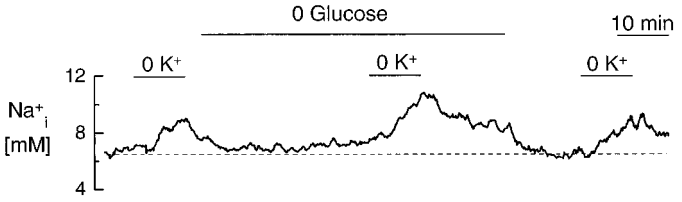

Figure 5. Influence of glucose removal on $[Na^+]_i$ increases induced by removal of $[K^+]_e$. Removal of $[K^+]_e$ (10 min; indicated by *short horizontal bars*) induced a small increase in $[Na^+]_i$ attributable to inhibition of Na^+/K^+-ATPase. The recovery from this $[Na^+]_i$ increase was not significantly influenced by glucose removal (*long horizontal bar*).

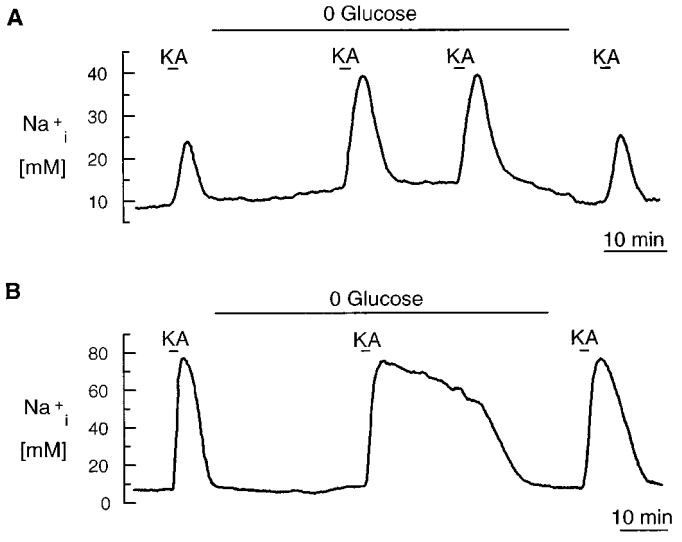

Figure 6. Influence of glucose removal on large $[Na^+]_i$ increases induced by kainate. Application of the glutamatergic agonist kainate (*short horizontal bars* marked *KA*; 0.5 mM) induced rapid increases in $[Na^+]_i$ in ~30% of the spinal cord astrocytes. In cells in which these $[Na^+]_i$ increases were larger than ~15 mM, removal of glucose (*long horizontal bars*) either caused an increase in their amplitude (*A*) or significantly attenuated $[Na^+]_i$ recovery (*B*), indicating that the presence of glucose was necessary to ensure efficient Na^+ extrusion from the astrocytes.

crease in baseline $[Na^+]_i$ did not hamper the ability of the cell to recover from small $[Na^+]_i$ loads.

Kainate (0.5 mM; 2 min) changed $[Na^+]_i$ in 39% ($n = 184$ total) of spinal cord astrocytes by opening a cation channel coupled to a non-NMDA receptor (Rose and Ransom, 1996b; Rose et al., 1997). As reported earlier (Rose et al., 1997), kainate decreased $[Na^+]_i$ by ~2 mM in 21% of the responding cells and variably increased $[Na^+]_i$ in the remainder (average increase, 13.3 ± 10.9 mM). Removal of glucose or application of NaN_3 and 0 glucose did not change kainate-induced decreases in $[Na^+]_i$, nor did it change kainate-induced increases in $[Na^+]_i$, if they were small (i.e., <15 mM; data not shown).

Kainate-induced $[Na^+]_i$ increases of >15 mM, in contrast, were augmented in amplitude (increase, 45% larger than control; $n = 3$; Fig. 6*A*) or slowed in recovery [average recovery time, 29.0 ± 7.2 min ($n = 5$) vs 7.6 ± 4.8 min under control conditions ($n = 56$)] in 0 glucose (Fig. 6*B*). Recovery from kainate-induced $[Na^+]_i$ increases of >15 mM was also significantly prolonged when oxidative phosphorylation was inhibited by NaN_3 (recovery time, 24.3 ± 6.4 min; $n = 5$; Fig. 7*A*). No recovery at all was observed during metabolic inhibition with NaN_3 and 0 glucose ($n = 26$; Fig. 7*B*).

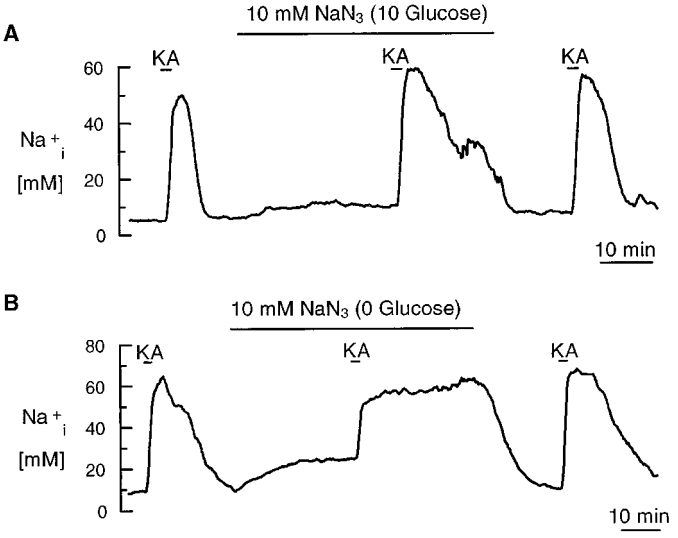

Figure 7. Influence of chemical hypoxia on large, kainate-induced $[Na^+]_i$ increases. *A*, Inhibition of oxidative phosphorylation by perfusion with 10 mM NaN_3 in the presence of glucose (indicated by *long horizontal bar*) significantly delayed recovery from large, kainate-induced increases in $[Na^+]_i$. *B*, Inhibition of both glycolysis and oxidative phosphorylation by glucose-free saline containing 10 mM NaN_3 (*long horizontal bar*) completely inhibited recovery from kainate-induced increases in $[Na^+]_i$. Kainate was applied at 0.5 mM for 2 min as indicated by the *short horizontal bars* marked *KA*.

These results allow several conclusions about $[Na^+]_i$ homeostasis in spinal cord astrocytes during metabolic inhibition for 60 min. During inhibition of glycolysis or oxidative phosphorylation, $[Na^+]_i$ regulation remained functional, and despite a slight increase in baseline $[Na^+]_i$, the cells were able to extrude additional, small Na^+ loads. Recovery from $[Na^+]_i$ increases larger than ~15 mM, however, was significantly impaired under these conditions, suggesting that neither of the two affected pathways (glycolysis and oxidative phosphorylation) was sufficient alone to ensure optimal recovery from large $[Na^+]_i$ loads. Astrocytes completely lost their ability to extrude Na^+ during total energy metabolism blockade caused by both glucose removal and inhibition of oxidative phosphorylation.

$[Na^+]_i$ changes caused by simulated ischemia

Pathological insults such as anoxia and ischemia are characterized by dramatic changes in the ion composition of the extracellular space in the brain (Hansen, 1985; Ransom et al., 1992; Erecinska and Silver, 1994; Kraig et al., 1995). These changes include an increase in $[K^+]_e$ and a decrease in $[Na^+]_e$, $[Ca^{2+}]_e$, and pH_e. In addition, extracellular lactate as well as extracellular glutamate concentrations rise strongly (Shimada et al., 1993). To mimic these ischemic conditions *in vitro*, we exposed astrocytes to a glucose-free saline containing 10 mM NaN_3, 60 mM K^+, 95 mM Na^+, 0.2 mM Ca^{2+}, 20 mM L-lactate, 0.1 mM glutamate, and 20 mM PIPES (1,4-piperazinediethanesulfonic acid), titrated to a pH of 6.5, called "ischemia solution" (cf. Hansen, 1985; Ransom et al., 1992; Shimada et al., 1993; Erecinska and Silver, 1994; Kraig et al., 1995).

A 60 min perfusion with this solution resulted in multiphasic changes in $[Na^+]_i$ ($n = 81$; see Figs. 8, 11). At the onset of the simulated ischemia, $[Na^+]_i$ quickly dropped by 4.0 ± 4.9 mM. After 10–15 min, the $[Na^+]_i$ drop was followed by a slow increase in $[Na^+]_i$ to a value 2.9 ± 3.1 mM higher than the original baseline

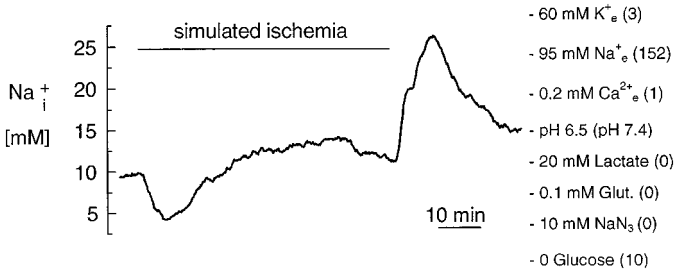

Figure 8. $[Na^+]_i$ changes induced by simulated ischemia. To mimic anoxic and ischemic conditions in the brain, the astrocytes were perfused with a saline with altered ionic composition (listed on the *right*; the concentrations used in standard saline are indicated in *parentheses*) for 1 hr (*horizontal bar* marked *simulated ischemia*). *Simulated ischemia* resulted in multiphasic changes in $[Na^+]_i$, with the most prominent $[Na^+]_i$ transient occurring after reperfusion with standard saline.

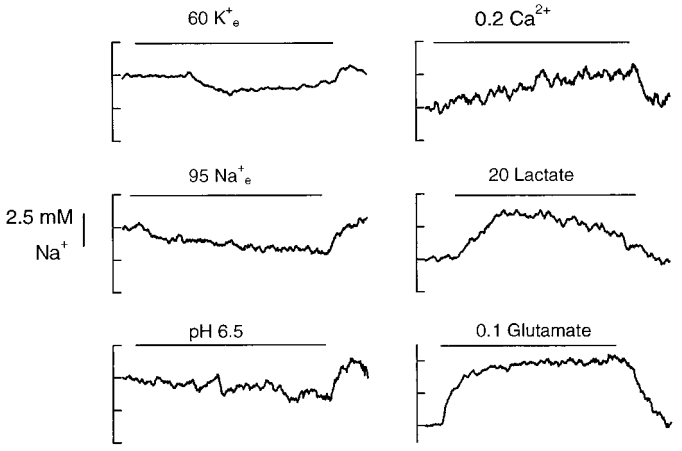

Figure 9. $[Na^+]_i$ changes induced by the single components of simulated ischemia. To analyze the effects of simulated ischemia on $[Na^+]_i$, we investigated the effects of each of the individual manipulations. Shown are absolute changes in $[Na^+]_i$; the manipulations were performed for 60 min as indicated by the *horizontal bars*. All manipulations caused monophasic and completely reversible changes in $[Na^+]_i$. Increasing $[K^+]_e$ to 60 mM while reducing $[Na^+]_e$ to 95 mM or reducing $[Na^+]_e$ alone caused $[Na^+]_i$ to decrease by <3 mM (*upper* and *middle* on the *left*). In a few cells, an acidification to pH 6.5 resulted in a minor fall in $[Na^+]_i$ (<2 mM; *lower* on the *left*). All other changes to the standard saline induced elevations in $[Na^+]_i$ (*right*); these changes are reduction of $[Ca^{2+}]_e$ to 0.2 mM (*upper*), addition of 20 mM L-lactate (*middle*), and addition of 0.1 mM glutamate (*lower*).

in standard saline. The most dramatic change in $[Na^+]_i$, however, took place shortly after reperfusion with standard saline, when $[Na^+]_i$ rapidly increased by another 7.6 ± 7.1 mM. In contrast to the effects observed with NaN_3 and 0 glucose, which were completely reversible within 10–15 min (compare Fig. 3*A*), the astrocytes recovered more slowly from the ischemia solution-induced $[Na^+]_i$ increase, and $[Na^+]_i$ was still elevated by ~4 mM after a 30 min reperfusion with standard saline (Fig. 8).

To analyze the mechanisms underlying these multiphasic $[Na^+]_i$ changes, we investigated the individual effects of each ionic alteration made in the ischemia solution. Perfusion with 60 mM K^+ and 95 mM Na^+ (n = 36), or with 95 mM Na^+ (n = 53; Na^+ replaced by *N*-methyl-D-glucamine) caused a decrease in $[Na^+]_i$ by <3 mM (see Figs. 9, 11). Decreasing pH_e to 6.5 caused a small, reversible fall in $[Na^+]_i$ (<2 mM) in 9 out of 41 cells investigated (Fig. 9). All other changes to the standard saline-induced elevations in $[Na^+]_i$ are as follows (see Figs. 9, 11): 0.2

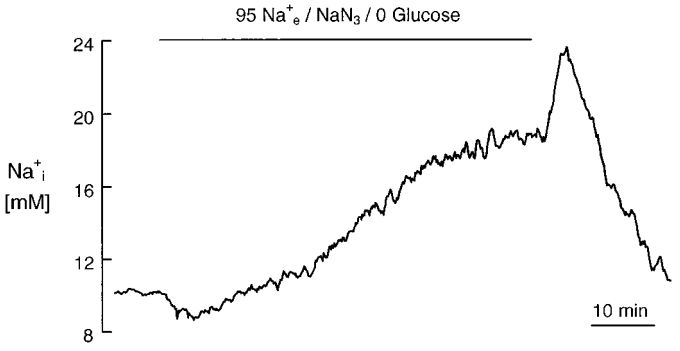

Figure 10. $[Na^+]_i$ changes induced by a reduction in $[Na^+]_e$ combined with metabolic inhibition. The combined reduction in $[Na^+]_e$ to 95 mM and metabolic inhibition by NaN_3 and 0 glucose for 1 hr (indicated by *horizontal bar*) resulted in multiphasic $[Na^+]_i$ changes, which were similar to those induced by simulated ischemia.

mM $[Ca^{2+}]_e$ increased $[Na^+]_i$ by ~2 mM (n = 38), 20 mM lactate increased $[Na^+]_i$ by ~4 mM (n = 55), and 0.1 mM glutamate increased $[Na^+]_i$ by ~5 mM (n = 53). Removal of glucose or addition of NaN_3 increased $[Na^+]_i$ as described above (compare Figs. 2, 3).

Each of the $[Na^+]_i$ changes seen with individual ionic or chemical alterations was monophasic and completely reversible within 10–15 min after switching back to standard saline. Multiphasic $[Na^+]_i$ changes mimicking those observed with simulated ischemia, however, were obtained when the astrocytes were perfused with a saline containing NaN_3, 0 glucose, and 95 mM Na^+ (n = 61; Figs. 10, 11); an initial decrease in $[Na^+]_i$ was followed by a slow $[Na^+]_i$ increase that was followed by a rapid secondary increase in $[Na^+]_i$ when switching back to normal saline. In contrast to the situation found with simulated ischemia, however, recovery from this secondary $[Na^+]_i$ increase was usually complete within 10–15 min (Fig. 10). These results suggest that the reduction in $[Na^+]_e$ that accompanies inhibition of metabolism in ischemia (Hansen, 1985) may be of special importance as an influence on $[Na^+]_i$ in spinal cord astrocytes during ischemia.

DISCUSSION

Mechanisms of $[Na^+]_i$ increases during inhibition of energy production

The effects of various forms of metabolic inhibition on $[Na^+]_i$ regulation in cultured rat spinal cord astrocytes were analyzed in this study. Only small increases in $[Na^+]_i$ (<10 mM) were observed when either glycolysis or oxidative respiration alone were inhibited for 60 min. This is consistent with the effects of such inhibition on astrocyte [ATP]. In general, blocking either aerobic or glycolytic metabolism has little effect on [ATP] (Pauwels et al., 1985; Gregory et al., 1990; Kelleher et al., 1993; Gemba et al., 1994; Hori et al., 1994; Longuemare et al., 1994; for review, see Silver and Erecinska, 1997). The fact that blocking oxidative metabolism alone, as seen with NaN_3, does not have a strong effect on [ATP] and, for that reason, on $[Na^+]_i$ is a consequence of the high glycolytic rate of astrocytes (Silver and Erecinska, 1997). The minimal effect of 0 glucose on [ATP] is easily explained on the basis of their stored glycogen that can generate glucose residues. The capacity of astrocytes to maintain near normal [ATP] when glycolysis is blocked is probably attributable to their ability to metabolize ketone bodies and Krebs cycle intermediates (Wiesinger et al., 1997). It has been postulated that the ATP produced by glycolysis selectively supports Na^+/K^+-

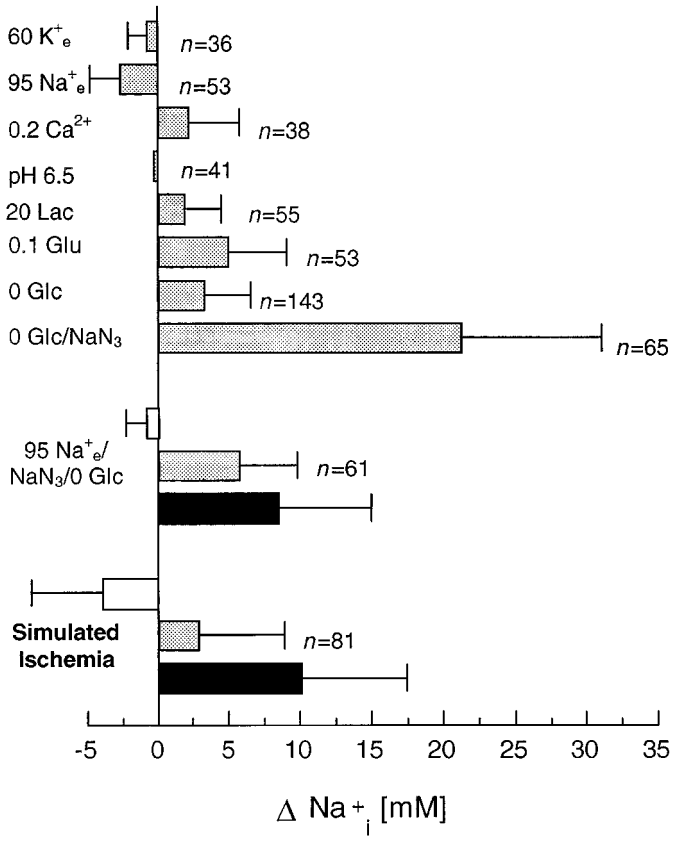

Figure 11. Summary of $[Na^+]_i$ changes induced by simulated ischemia. The mean values ± SD of $[Na^+]_i$ changes (ΔNa^+_i) measured after 60 min of perfusion with the corresponding salines (*gray columns*) and the number of cells investigated (*n*) are shown on the histogram. Negative and positive values represent decreases and increases in $[Na^+]_i$, respectively, compared with baseline $[Na^+]_i$ in standard saline. Although changing single parameters caused monophasic $[Na^+]_i$ changes (first eight *gray columns*), the combined reduction in $[Na^+]_e$ with application of NaN_3 and 0 glucose as well as simulated ischemia resulted in multiphasic changes in $[Na^+]_i$; small $[Na^+]_i$ decreases (*white columns*) turned into slow $[Na^+]_i$ increases (*gray columns*), which were followed by more rapid $[Na^+]_i$ increases when switching back to standard saline (*black columns*).

ATPase (Lipton and Robacker, 1983; Kauppinen et al., 1988; Raffin et al., 1988). Our observations that glycolytic blockade minimally changed $[Na^+]_i$ is not consistent with this assertion.

In contrast to the small changes in [ATP] that occur with isolated inhibition of glycolysis or oxidative respiration, [ATP] drops within 5–10 min to near 0 when both are inhibited (Longuemare et al., 1994; Silver at al., 1997). The 20 mM increase in astrocytic $[Na^+]_i$ observed after 60 min of combined glycolytic and respiratory blockade most likely resulted from complete failure of Na^+/K^+-ATPase (Lees, 1991). Consistent with this, we observed a very similar $[Na^+]_i$ increase when Na^+/K^+-ATPase was inhibited by ouabain. These results indicate a very low leakage of sodium ions of spinal cord astrocytes.

Relatively moderate $[Na^+]_i$ increases during metabolic inhibition were also reported in mouse astrocytes (Silver et al., 1997) and rat Schwann cells (Lehning et al., 1995), whereas $[Na^+]_i$ rose to near 140 mM in cultured rat cortical astrocytes after 45 min of combined glycolytic and respiratory blockage (M. C. Longuemare, C. R. Rose, B. R. Ransom, S. G. Waxman, and R. A. Swanson, unpublished observations). These differences point to differential susceptibility of astrocytes derived from different brain regions to metabolic failure, similar to the situation with neurons (Auer and Siesjö, 1988) and echo a common theme in astrocyte physiology (Ransom, 1991). Neuronal $[Na^+]_i$ changes may be larger than those in glial cells, however, when studied in the same preparation (Lehning et al., 1995; LoPachin and Stys, 1995). In cultured CA1-hippocampal neurons, $[Na^+]_i$ increased by 27 mM after only 3–4 min of anoxia (Friedman and Haddad, 1994), emphasizing the high susceptibility of these neurons to hypoglycemic and anoxic damage (Auer and Siesjö, 1988).

Glia are less susceptible to injury caused by metabolic inhibition than are neurons (Goldberg and Choi, 1993). This might be related to less Na^+ influx in glia. Our investigation shows that despite their relatively high density (Sontheimer et al., 1992), voltage-gated Na^+ channels probably do not mediate Na^+ influx into spinal cord astrocytes during glycolytic and respiratory inhibition. In neurons, in contrast, TTX can provide significant protection from anoxic damage (Stys et al., 1992; Lynch et al., 1995; Agrawal and Fehlings, 1996; Lehning et al., 1996; Imaizumi et al., 1997). Astrocytes, in addition, probably experience less glutamate-gated Na^+ influx than do neurons [Auer and Siesjö (1988); Choi and Rothman (1990); see, however, David et al. (1996)]. Consequently, harmful Ca^{2+} influx caused by reversal of Na^+–Ca^{2+} exchange (Waxman et al., 1991; Ransom and Fern, 1996) might be smaller in astrocytes because of less Na^+ entry.

Na^+ regulatory capacity during metabolic inhibition

The small $[Na^+]_i$ increases observed in spinal cord astrocytes with prolonged inhibition of glycolysis or respiration did not greatly reduce the transmembrane Na^+ gradient. The astrocytes were still able to recover from small additional Na^+ loads, indicating that [ATP] was sufficient to allow an increase in Na^+/K^+-ATPase activity. Challenged with large $[Na^+]_i$ loads (>15 mM), however, recovery was attenuated, indicating that maximal glial Na^+/K^+-ATPase activity was limited during metabolic inhibition. In the face of attenuated $[Na^+]_i$ recovery, the capacity to recover from accompanying changes in other ions would also be reduced, resulting in a general disturbance of cellular ion regulation as described for neurons (Kiedrowski et al., 1994; Koch and Barish, 1994).

The maintenance of high E_{Na} even after 1 hr of combined glycolytic and respiratory blockage suggests that other ion gradients may be maintained as well; consistent with this prediction, relatively small changes in $[Ca^{2+}]_i$ have been reported from acutely isolated hippocampal astrocytes during these insults (Duffy and MacVicar, 1996). Other changes observed in astrocytes during glycolytic and respiratory failure include slow membrane depolarization, K^+ loss, acidification, and swelling (Kimelberg and Ransom, 1986; Kauppinen et al., 1988; Kraig and Chesler, 1990; Walz et al., 1993).

Despite the high E_{Na} maintained during metabolic failure, active $[Na^+]_i$ regulation failed in spinal cord astrocytes when they were challenged by large additional $[Na^+]_i$ loads [see also Lees (1991); Erecinska and Silver (1994)]. These results suggest that the transmembrane Na^+ gradient and capacity for intracellular ion regulation would be lost rapidly during metabolic failure in intact tissue if astrocytes experience heavy Na^+ loading. This, in fact, was reported by Duffy and MacVicar (1996), who showed that the ischemia-induced $[Ca^{2+}]_i$ increase was larger in astrocytes within slices than in isolated cells.

Mechanisms of $[Na^+]_i$ changes caused by simulated ischemia

Ischemia-induced energy failure results in marked alterations of extracellular ion concentrations in intact tissue. In gray matter, $[K^+]_e$ and $[Na^+]_e$ change to ~60 mM, $[Ca^{2+}]_e$ decreases to 0.2 mM, pH_e decreases to 6.5, and extracellular lactate and glutamate concentrations increase (Hansen, 1985; Erecinska and Silver, 1994; Sykova et al., 1994; Kraig et al., 1995). In white matter, extracellular ion changes are qualitatively similar but smaller in amplitude (Ransom et al., 1992).

Our results show that no one of these extracellular changes caused the sequence of $[Na^+]_i$ change in spinal cord astrocytes that was characteristic of metabolic inhibition. The small decrease in $[Na^+]_i$ observed with elevated $[K^+]_e$ was probably attributable to activation of Na^+/K^+-ATPase and cell swelling (Walz and Hertz, 1983; Rose and Ransom, 1996a), whereas the $[Na^+]_i$ decrease during low $[Na^+]_e$ was probably caused by reduction of the Na^+ gradient (Rose and Ransom, 1996a). The reason for the small $[Na^+]_i$ increase induced by $[Ca^{2+}]_e$ reduction is unclear, but it could have been caused by altered Na^+–Ca^{2+} exchange (Goldman et al., 1994). Acidification did not alter $[Na^+]_i$ significantly, whereas lactate caused a sustained increase in $[Na^+]_i$, consistent with activation of a Na^+-dependent uptake mechanism in astrocytes (Walz and Mukerji, 1988). Glutamate, applied at a concentration highly toxic for neurons (Choi and Rothman, 1990), caused a small (~5 mM), sustained $[Na^+]_i$ increase that was probably related to Na^+-coupled uptake (Rose and Ransom, 1996b).

Applying all these ionic changes in conjunction with glycolytic and respiratory inhibition (simulated ischemia, Fig. 8) revealed that $[Na^+]_i$ changes caused by single manipulations were not simply additive. Instead, simulated ischemia resulted in multiphasic $[Na^+]_i$ changes that could only be mimicked by subjecting the astrocytes to metabolic failure and decreased $[Na^+]_e$. This combination led to a small decrease in $[Na^+]_i$ at the beginning of the insult and limited the strong $[Na^+]_i$ increase observed during metabolic failure alone, presumably because of the Na^+ gradient reduction combined with cell swelling. The largest $[Na^+]_i$ change, however, occurred after reperfusion with standard saline when the normal $[Na^+]_e$ was restored. This phenomenon, which has not been described before, suggests that the reinstatement of [ATP] and Na^+/K^+-ATPase activity lagged behind the re-establishment of the Na^+ gradient.

An analogous reperfusion-mediated phenomenon reported in astrocytes is a strong rise in $[Ca^{2+}]_i$ subsequent to perfusion with Ca^{2+}-free saline; this so-called "reperfusion paradox" is attributable to elevation of $[Na^+]_i$ via Na^+-permeable Ca^{2+} channels, followed by reverse Na^+–Ca^{2+} exchange (Kim-Lee et al., 1992). These events, the rebound elevation of $[Na^+]_i$ and the calcium paradox, suggest that astrocytes might be most susceptible to ischemic damage during the reperfusion period.

Possible role of astrocytes during metabolic insult

Despite a reduction in their capacity for Na^+ extrusion, cultured spinal cord astrocytes maintain a steep inwardly directed Na^+ gradient during a 60 min period of energy metabolism inhibition. This is true even during combined glycolytic and respiratory failure, although Na^+/K^+-ATPase-mediated Na^+ export is entirely blocked. The Na^+ gradient may be even better protected during *in vivo* ischemia because of extracellular ion changes. With a concomitant reduction in $[Na^+]_e$, the astrocytes can maintain their Na^+ gradient close to normal even during prolonged periods of complete energy failure, possibly enabling them to maintain Na^+-dependent ion regulation. Because Na^+/K^+-ATPase activity is blocked, any additional Na^+ loads, e.g., attributable to channel-mediated influx, will, however, decrease the Na^+ gradient. This would be expected to promote reversal of Na^+-dependent plasma membrane carriers. We predict that, in intact tissue, astrocytes may protect neurons at the onset of ischemic insults by virtue of their continued capacity for regulation of extracellular ion concentrations and glutamate uptake but may aggravate damage by release of acid, K^+, and/or glutamate as a result of compromised $[Na^+]_i$ extrusion because of energy failure during long-lasting ischemia.

REFERENCES

Agrawal SK, Fehlings MG (1996) Mechanisms of secondary injury to spinal cord axons *in vitro*: role of Na^+, Na^+/K^+-ATPase, the Na^+-H^+ exchanger, and the Na^+-Ca^{2+} exchanger. J Neurosci 16:545–552.

Ames III A, Li Y-Y, Heher EC, Kimble CR (1992) Energy metabolism of rabbit retina as related to function: high cost of Na^+ transport. J Neurosci 12:840–853.

Auer RN, Siesjö BK (1988) Biological differences between ischemia, hypoglycemia, and epilepsy. Ann Neurol 24:699–707.

Billups B, Attwell D (1996) Modulation of non-vesicular glutamate release by pH. Nature 379:171–174.

Black JA, Sontheimer H, Waxman SG (1993) Spinal cord astrocytes *in vitro*: phenotypic diversity and sodium channel immunoreactivity. Glia 7:272–285.

Choi DW, Rothman SM (1990) The role of glutamate neurotoxicity in hypoxic–ischemic neuronal death. Annu Rev Neurosci 13:171–182.

David JC, Yamada KA, Bagwe MR, Goldberg MP (1996) AMPA receptor activation is rapidly toxic to cortical astrocytes when desensitization is blocked. J Neurosci 16:200–209.

Duffy S, MacVicar BA (1996) *In vitro* ischemia promotes calcium influx and intracellular calcium release in hippocampal astrocytes. J Neurosci 16:71–81.

Erecinska M, Silver IA (1994) Ions and energy in mammalian brain. Prog Neurobiol 43:37–71.

Friedman JE, Haddad GG (1994) Anoxia induces an increase in intracellular sodium in rat central neurons *in vitro*. Brain Res 663:329–334.

Gemba T, Oshima T, Nimomiya M (1994) Glutamate efflux via the reversal of the sodium-dependent glutamate transporter caused by glycolytic inhibition in rat cultured astrocytes. Neuroscience 63:789–795.

Goldberg MP, Choi DW (1993) Combined oxygen and glucose deprivation in cortical cell culture: calcium-dependent and calcium-independent mechanisms of neuronal injury. J Neurosci 13:3510–3524.

Goldman WF, Yarowsky PJ, Juhaszova M, Krueger BK, Blaustein MP (1994) Sodium/calcium exchange in rat cortical astrocytes. J Neurosci 14:5834–5843.

Gregory GA, Welsh FA, Yu ACH, Chan PH (1990) Fructose-1,6-bisphosphate reduces ATP loss from hypoxic astrocytes. Brain Res 516:310–312.

Hamprecht B, Dringen R (1995) Energy metabolism. In: Neuroglia (Kettenmann H, Ransom BR, eds), pp 473–487. New York: Oxford UP.

Hansen AJ (1985) Effect of anoxia on ion distribution in the brain. Physiol Rev 65:101–148.

Hori O, Matsumoto M, Maeda Y, Ueda H, Ohtsuki T, Stern DM, Kinoshita T, Ogawa S, Kamada T (1994) Metabolic and biosynthetic alterations in cultured astrocytes exposed to hypoxia/reoxygenation. J Neurochem 62:1489–1495.

Imaizumi T, Kocsis JD, Waxman SG (1997) Anoxic injury in rat spinal cord: pharmacological evidence for multiple steps in Ca-dependent injury of the dorsal columns. J Neurotrauma 14:299–313.

Kauppinen RA, Enkvist K, Holopainen I, Akerman KEO (1988) Glucose deprivation depolarizes plasma membrane of cultured astrocytes and collapses transmembrane potassium and glutamate gradients. Neuroscience 26:283–289.

Kelleher SA, Chan PN, Chan TY, Gregory GA (1993) Modification of hypoxia-induced injury in cultured rat astrocytes by high levels of glucose. Stroke 24:855–863.

Kiedrowski L, Brooker G, Costa E, Wroblewski JT (1994) Glutamate impairs neuronal calcium extrusion while reducing sodium gradient. Neuron 12:295–300.

Kim-Lee MH, Stokes BT, Yates AJ (1992) Reperfusion paradox: a novel mode of glial cell injury. Glia 5:56–64.

Kimelberg HK, Ransom BR (1986) Physiological and pathological aspects of astrocytic swelling. In: Astrocytes (Federoff S, Vernadakis A, eds), pp 129–166. Orlando, FL: Academic.

Kimelberg HK, Pang S, Treble DH (1989) Excitatory amino acid-stimulated uptake of $^{22}Na^+$ in primary astrocyte cultures. J Neurosci 9:1141–1149.

Koch RA, Barish ME (1994) Perturbation of intracellular calcium and hydrogen ion regulation in cultured mouse hippocampal neurons by reduction of the sodium ion concentration gradient. J Neurosci 14:2585–2593.

Kraig RP, Chesler M (1990) Astrocytic acidosis in hyperglycemic and complete ischemia. J Cereb Blood Flow Metab 10:104–114.

Kraig RP, Lascola CD, Caggiano A (1995) Glial response to brain ischemia. In: Neuroglia (Kettenmann H, Ransom BR, eds), pp 964–976. New York: Oxford UP.

Lees GJ (1991) Inhibition of sodium–potassium-ATPase: a potentially ubiquitous mechanism contributing to central nervous system neuropathology. Brain Res Rev 16:283–300.

Lehning EJ, Doshi R, Stys PK, LoPachin RM (1995) Mechanisms of injury-induced calcium entry into peripheral nerve myelinated axons: *in vitro* anoxia and ouabain exposure. Brain Res 694:158–166.

Lehning EJ, Doshi R, Isaksson N, Stys PK, LoPachin RM (1996) Mechanisms of injury-induced calcium entry into peripheral nerve myelinated axons: role of reverse sodium–calcium exchange. J Neurochem 66:493–500.

Lipton P, Robacker K (1983) Glycolysis and brain function: $[K^+]_o$ stimulation of protein synthesis and K^+ uptake require glycolysis. Fed Proc 42:2875–2880.

Longuemare MC, Hill MP, Swanson RA (1994) Glycolysis can prevent non-synaptic excitatory amino acid release during hypoxia. NeuroReport 5:1789–1792.

LoPachin RM, Stys PK (1995) Elemental composition and water content of rat optic nerve myelinated axons and glial cells: effects of *in vitro* anoxia and reoxygenation. J Neurosci 15:6735–6746.

Lucas JH, Emery DG, Rosenberg LJ (1997) Physical injury of neurons: important roles for sodium and chloride ions. The Neuroscientist 3:89–101.

Lynch III JJ, Yu SP, Canzoniero LM, Sensi SL, Choi DW (1995) Sodium channel blockers reduce oxygen-glucose deprivation-induced cortical neuronal injury when combined with glutamate receptor antagonists. J Pharmacol Exp Ther 273:554–560.

Pappas CA, Rioult MG, Ransom BR (1996) Octanol, a gap junction uncoupling agent, changes intracellular $[H^+]$ in rat astrocytes. Glia 16:7–15.

Pauwels PJ, Opperdoes FR, Trouet A (1985) Effects of antimycin, glucose deprivation, and serum on cultures of neurons, astrocytes, and neuroblastoma cells. J Neurochem 44:143–148.

Raffin CN, Sick TJ, Rosenthal M (1988) Inhibition of glycolysis alters potassium ion transport and mitochondrial redox activity in rat brain. J Cereb Blood Flow Metab 8:857–865.

Ransom BR (1991) Vertebrate glial classification, lineage, and heterogeneity. In: Glial–neuronal interactions (Abott NJ, ed), pp 19–26. New York: Ann NY Acad Sci.

Ransom BR, Fern R (1996) Anoxic–ischemic glial cells injury: mechanisms and consequences. In: Tissue oxygen deprivation (Haddad GG, Lister G, eds), pp 617–652. New York: Dekker.

Ransom BR, Walz W, Davis PK, Carlini WG (1992) Anoxia-induced changes in extracellular K^+ and pH in mammalian central white matter. J Cereb Blood Flow Metab 12:593–602.

Rose CR, Ransom BR (1996a) Intracellular Na^+ homeostasis in cultured rat hippocampal astrocytes. J Physiol (Lond) 491:291–305.

Rose CR, Ransom BR (1996b) Mechanisms of H^+ and Na^+ changes induced by glutamate, kainate, and D-aspartate in rat hippocampal astrocytes. J Neurosci 16:5393–5404.

Rose CR, Ransom BR, Waxman SG (1997) Pharmacological characterization of Na^+ influx via voltage-gated Na^+ channels in spinal cord astrocytes. J Neurophysiol 87:3249–3258.

Rosenberg PA (1991) Accumulation of extracellular glutamate and neuronal death in astrocyte-poor cultures exposed to glutamine. Glia 4:91–100.

Shimada N, Graf R, Rosner G, Heiss WD (1993) Ischemia-induced accumulation of extracellular amino acids in cerebral cortex, white matter, and cerebrospinal fluid. J Neurochem 60:66–71.

Siesjö BK (1985) Membrane events leading to glial swelling and brain edema. In: Brain edema (Inaba Y, ed), pp 200–209. Berlin: Springer.

Silver IA, Erecinska M (1997) Energetic demands of the Na^+/K^+ ATPase in mammalian astrocytes. Glia 21:35–45.

Silver IA, Deas J, Erecinska M (1997) Ion homeostasis in brain cells: differences in intracellular ion responses to energy limitation between cultured neurons and glial cells. Neuroscience 78:589–601.

Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The $[^{14}C]$ deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916.

Sontheimer H, Black JA, Ransom BR, Waxman SG (1992) Ion channels in spinal cord astrocytes *in vitro*. I. Transient expression of high levels of Na+ and K+ channels. J Neurophysiol 68:985–1000.

Stys PK, Waxman, SG, Ransom BR (1992) Ionic mechanisms of anoxic injury in CNS white matter: role of Na^+ channels and Na^+-Ca^{2+} exchanger. J Neurosci 12:430–439.

Swanson RA (1992) Astrocyte glutamate uptake during chemical hypoxia *in vitro*. Neurosci Lett 147:143–146.

Swanson RA, Choi DW (1993) Glial glycogen stores affect neuronal survival during glucose deprivation *in vitro*. J Cereb Blood Flow Metab 13:162–169.

Sykova E, Svoboda J, Polak J, Chvatal A (1994) Extracellular volume fraction and diffusion characteristics during progressive ischemia and terminal anoxia in the spinal cord of the rat. J Cereb Blood Flow Metab 14:301–311.

Tator CH, Fehlings MG (1991) Review of the secondary theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 75:15–26.

Tsacopoulos M, Magistretti PJ (1996) Metabolic coupling between glia and neurons. J Neurosci 16:877–885.

Vibulsreth S, Hefti F, Ginsberg MD, Dietrich WD, Busto R (1987) Astrocytes protect cultured neurons from degeneration induced by anoxia. Brain Res 422:330–311.

Walz W, Hertz L (1982) Ouabain-sensitive and ouabain-resistant uptake of potassium into astrocytes and neurons in primary culture. J Neurochem 39:70–77.

Walz W, Hertz L (1983) Intracellular ion changes of astrocytes in response to extracellular potassium. J Neurosci Res 10:411–423.

Walz W, Mukerji S (1988) Lactate production and release in cultured astrocytes. Neurosci Lett 86:296–300.

Walz W, Klimaszewski A, Paterson IA (1993) Glial swelling in ischemia: a hypothesis. Dev Neurosci 15:216–225.

Waxman SG, Ransom BR, Stys PK (1991) Non-synaptic mechanisms of Ca^{2+}-mediated injury in CNS white matter. Trends Neurosci 14:461–468.

Wiesinger B, Hamprecht B, Dringen R (1997) Metabolic pathways for glucose in astrocytes. Glia 21:22–34.