Inhibition of the Electrogenic Na Pump Underlies Delayed Depolarization of Cortical Neurons After Mechanical Injury or Glutamate

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Tavalin, Steven J., Earl. F. Ellis, and Leslie S. Satin. Inhibition of the electrogenic Na pump underlies delayed depolarization of cortical neurons after mechanical injury or glutamate. J. Neurophysiol. 77: 632–638, 1997. We previously characterized the electrophysiological response of cortical neurons to a brief sublethal stretch-injury using an in vitro model of traumatic brain injury. This model revealed that cortical neurons undergo a stretch-induced delayed depolarization (SIDD) of their resting membrane potential (RMP) which is ~10 mV in magnitude. SIDD is dependent on N-methyl-D-aspartate (NMDA) receptor activation, neuronal firing, and extracellular calcium for its induction but not its maintenance. SIDD was maximal 1 h after the insult and required incubation at 37°C. The present study examined the mechanism mediating SIDD and its relation to glutamate receptor activation. The Na pump inhibitor ouabain was used to assess the contribution of the Na pump to the RMP of control and stretched neurons using whole cell patch-clamp techniques. The nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine and a polyethylene glycol conjugate of superoxide dismutase were used to assess whether NO or superoxide anion, respectively, were involved in the induction of SIDD. Neurons were exposed to exogenous glutamate in the absence of potential (RMP) of stretch-injured neurons was depolarized by ~10 mV. This depolarization required a 1-h incubation period after the mechanical insult and subsided by 24 h. We report that SIDD is mediated by Na pump inhibition and is likely to result from reduced energy levels since the RMP of neurons dialyzed with a pipette solution containing 5 mM ATP were identical to controls. NO, but not superoxide anion, also may contribute to SIDD. A 3-min exposure to 10 μM glutamate produced a SIDD-like depolarization also associated with Na pump inhibition. The results suggest that Na pump inhibition secondary to alterations in cellular energetics underlies SIDD. Na pump inhibition due to glutamate exposure may contribute to traumatic brain injury or neurodegenerative diseases linked to glutamate receptor activation.

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The electrogentic Na/K-ATPase (Na pump) plays an important role in ionic homeostasis and tonically hyperpolarizes neurons due to ATP-dependent transport of Na⁺ and K⁺ (Glynn and Karlish 1975; Thomas 1972). The Na pump is coupled to cellular metabolic status as ~40% of the energy generated by respiration is used to maintain neuronal Na pump activity at rest (Astrup et al. 1981; Whittam 1962). Na pump activity contributes ~10 mV to neuronal RMP of sympathetic neurons (Jones 1989). Because TBI and glutamate excitotoxicity are associated with reductions in cellular energetic status (Ankarcrona et al. 1995; Faden et al. 1989; Retz and Coyle 1982; Rothman et al. 1987), we hypothesized that Na pump inhibition may contribute to SIDD. The
specific Na pump inhibitor ouabain was used to determine the contribution of the Na pump to the RMP of control and stretched neurons. We also voltage clamped neurons to measure directly ouabain-sensitive pump current.

Glutamate receptor activation is required for SIDD (Tavalin et al. 1995) and is known to be associated with calcium-dependent nitric oxide (NO) and superoxide anion production, both of which may contribute to excitotoxic neuronal injury (Coyle and Puttfarcken 1993; Dawson et al. 1992). Thus we hypothesized that NO and superoxide anion may contribute to SIDD. The nitric oxide synthase inhibitor Nω-nitro-L-arginine (L-NOARG) was used to assess whether inhibition of NO production prevents SIDD. Similarly, the polyethylene glycol conjugate of superoxide dismutase (PEG-SOD) was used to determine whether degradation of superoxide is protective. Last, we determined whether exogenous glutamate was capable of eliciting a SIDD-like depolarization in the absence of mechanical perturbation to test the hypothesis that mechanisms similar to SIDD also contribute to excitotoxicity.

We report that SIDD is mediated by Na pump inhibition, which is likely to be due to reduced energy levels because stretch-injured neurons recorded with 5 mM ATP within the patch pipette had RMPs identical to controls. Exposure of neurons to L-NOARG attenuated SIDD suggesting that NO may contribute to SIDD. In contrast, superoxide did not appear to contribute. Last, a 3-min application of 10 μM glutamate produced a neuronal depolarization that resembled SIDD-like depolarization in the absence of mechanical perturbation to test the hypothesis that mechanisms similar to SIDD also contribute to excitotoxicity.

METHODS

Cell culture

Primary cultures were prepared from 1- to 2-day-old Zivic-Miller rats as previously described (Tavalin et al. 1995). Briefly, neocortices were minced in saline and then trypsinized (0.125%) for 10 min at 37°C. Tissue was transferred to culture medium containing Dulbecco’s modified essential medium (DMEM) containing 4.5 g/l glucose supplemented with 10% fetal bovine serum, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (P/S). Cells were trituated and washed three times, counted, and plated at a density of 10⁴ cells per well in six-well Flex Plates (Flexcell International, McKeesport, PA). The bottom of each well was a 25-mm diam and 2-mm thick silastic membrane. These silastic membranes were collagen-coated before plating cells. Plates were incubated at 37°C in a 95%-5% mixture of air-carbon dioxide, with 95% humidity. Culture medium was replaced with growth medium (DMEM, 30 mM glucose, P/S, and 5% horse serum) 2–3 days after plating and cultures were fed twice weekly. Experiments were carried out at 12–16 days in vitro at which time the glial cell layer was near confluency.

Delivery of injury

Injury was delivered by use of a model 94A Cell Injury Controller (Commonwealth Biotechnology, Richmond, VA) as previously reported (Ellis et al. 1995; Tavalin et al. 1995). A single 50-ms pulse of compressed air was used to briefly deform the silastic membrane by 5.7 mm in all experiments. A 5.7-mm deformation is associated with a 31% stretch of the silastic membrane and adherent cells (Ellis et al. 1995). This level of injury is sublethal and reliably produces SIDD (Tavalin et al. 1995). Cells were stretched in normal external solution (see below). After the insult, cells were plated in a 95%-5% air-CO₂ incubator at 37°C for various times. Cells then were washed three times with standard external solution. Drug solutions were made from concentrated stock solutions and diluted in external solution. L-NOARG (Fluka, Ronkonkoma, NY) and PEG-SOD (Sanofi-Winthrop, Collegeville, PA) were applied 30 min before injury and as above cells were washed three times with standard external solution after incubation.

Electrophysiology

An Axopatch-1D (Axon Instruments, Forest City, CA) amplifier was used in tight-seal whole cell patch-clamp configuration (Hamil et al. 1981). Current- and voltage-clamp modes were used to measure the membrane potentials and currents of cultured cortical neurons. Patch pipettes (4–10 MΩ) were pulled from borosilicate glass (WPI, Sarasota, FL) using a two-stage puller (Model 750B, David Kopf Instruments, Tujunga, CA). Neurons were visualized using an Olympus IMT-2 inverted microscope at ×225. Phase-bright pyramidal neurons were selected for experiments. Recordings of these adherent neurons were made in situ. Cells were perfused continuously (1–2 ml/min) during recordings with standard external solution, which consisted of (in mM) 11.1 glucose, 130 NaCl, 5 KCl, 3 CaCl₂, 2 MgCl₂, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES; pH 7.20). The standard internal solution consisted of (in mM) 135 KOH, 135 aspartic acid, 5 KCl, 2 NaCl, 2 MgCl₂, 1 ethylene glycol-bis(β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid (EGTA), 0.2 CaCl₂, and 10 HEPES (pH 7.20). For the ATP-containing pipette solution, MgATP (Sigma, St. Louis, MO) was added and MgCl₂ was omitted. All recordings were made at room temperature (20°C). Some recordings were done in the presence of 1 μM tetrodotoxin (TTX; Sigma) to reduce spontaneous synaptic activity and neuronal firing.

Data acquisition and analysis

Data were acquired on a Macintosh Quadra 800 (Apple Computer, Cupertino, CA) using an Instrutech TIC-16 computer interface (Instrutech, Great Neck, NY) and Pulse Control (Herrington and Bookman, 1994) and Igor (Wavemetrics, Lake Oswego, OR) software. Currents and voltages were digitized and stored on VCR tape using an Instrutech VR-10-B digital data recorder. RMPs were determined within 1–3 min of establishing the whole cell configuration and were corrected for liquid junction potentials. Only cells showing stable resting potentials were included for analysis. No noticeable differences in sealing rates or successful establishment of the whole cell configuration were seen between control and stretched cells. Current-voltage (I-V) relationships were generated from a standard holding potential of ~60 mV using either test pulses (50–500 ms) or voltage ramps (10 mV/s). Input resistances at the 0 current potential (ZCP) were determined from linear regression of the I-V about the ZCP. RMPs measured in current clamp and ZCPs determined in voltage clamp were in excellent agreement. All data are expressed as the means ± SE.

RESULTS

Na pump contribution to SIDD

Current-clamp recordings from stretched and control neurons revealed that the RMP of stretched neurons were significantly depolarized compared to control (~51.1 ± 2.2 mV; n = 14 vs. ~63.4 ± 0.6 mV; n = 16, P < 0.01; Fig. 1A) as previously described (Tavalin et al. 1995). The
contribution of the electrogenic Na pump to cortical neuron RMP was assessed by bath application of the pump blocker ouabain. Ouabain (1 mM) depolarized control cells to a significantly greater extent than stretched cells (8.7 ± 1.5 mV; n = 7 vs. 2.6 ± 0.9 mV; n = 9, P < 0.01; Fig. 1B), suggesting that the Na pump already was inhibited by stretch injury. Ouabain depolarization was maximal within ~5–7 min of application and was in most cases fully reversible (not shown). Although ouabain is effective at blocking Na pump activity at much lower concentrations (Brines and Robbins 1992), this concentration was used to produce a relatively rapid effect given the slow association kinetics of the drug (Brines and Robbins 1992). Thus 100 μM ouabain also depolarized control cells by ~7 mV but this took closer to ~12 min (n = 2). Depolarization due to ouabain application often was accompanied by an increase in the frequency of excitatory postsynaptic potentials (EPSPs) and action potentials, presumably due to increased neurotransmitter release. However, it is unlikely that the ouabain depolarization was mediated by these excitatory events because ouabain depolarized neurons even in the presence of TTX (n = 2), which eliminated firing and large EPSPs. I–V relationships, obtained in voltage clamp, were quite variable but generally were found to be either linear over the range of voltages tested or showed inward rectification at potentials negative to ~80 mV. No noticeable differences in the shape of I–V curves between stretched and control neurons were detected. The input resistance of stretched and control cells about the M.O; n = 14 vs. 509 ± 71; n = 16, respectively). This indicates that the reduction of ouabain-induced depolarization observed in stretched neurons was not due to changes in pump current shunt conductance but in fact due to reduced pump current. Figure 1C shows that ouabain induced an inward pump current in voltage-clamped control neurons with no noticeable alteration in slope conductance. Figure 1D shows this pump current appears reduced in stretched cells. Na pump current, determined as the ouabain difference current at ~60 mV, in stretched neurons was reduced significantly compared with controls (6.3 ± 1.3 pA; n = 9 vs. 22.1 ± 7.1; n = 7, P < 0.05). Taken together these results indicate that neuronal stretch-injury is associated with reduced Na pump activity.

**Dependence of SIDD on intracellular ATP**

Various indices of cellular energetic status have been shown to decrease after TBI or excitotoxic insults (Ankarcrona et al. 1995; Faden et al. 1989; Rothman et al. 1987). To test whether Na pump inhibition was due to a reduction in cellular energy levels, ATP was supplied to the cell via the patch pipette. When ATP (5 mM) was included in the pipette solution, the RMPs of control and stretched neurons were no longer significantly different (~60.9 ± 1.1 mV; n = 16 vs. ~61.2 ± 1.0 mV; n = 17, respectively; Fig. 2A). Whole cell dialysis with 5 mM ATP had no effect on the RMP of control cells, suggesting that cellular energy levels were compromised only in stretched cells. In addition, ouabain depolarized stretched and control neurons to the same extent (8.3 ± 2.2 mV; n = 6 vs. 8.0 ± 1.8 mV; n = 6; Fig. 2B) when ATP was included in the pipette solution, implying that ATP restored the Na pump of stretched cells. The input resistances of stretched and control neurons under these conditions were not significantly different (525 ± 53 MΩ; n = 16 vs. 481 ± 49; n = 17), suggesting that the restoration of the ouabain-induced depolarization was by recovery of Na pump current rather than by changes in input resistance. In voltage clamp, ouabain was found to induce an inward current in control neurons dialyzed with 5 mM ATP without alteration of membrane slope conductance (Fig. 2C). In stretched neurons supplied with ATP, ouabain induced an inward current (Fig. 2D) much like that of controls. Na pump current was not significantly different in control and stretched neurons (26.1 ± 9.1 pA; n = 6 vs.
20.0 ± 7.0 pA; n = 6, respectively), indicating that the Na pump current of stretched cells could be recovered by pipette ATP. These results indicate that Na pump inhibition underlies SIDD and that pump inhibition is likely via a reduction in cellular energy levels.

**Contribution of nitric oxide and superoxide production to SIDD**

NO and superoxide anion production have been implicated in glutamate excitotoxicity (Coyle and Puttfarcken 1993; Dawson et al. 1992). Because both of these species have been reported to inhibit mitochondrial function, we hypothesized they might be a metabolic link between the activation of glutamate receptors following stretch and Na pump inhibition. To test whether either of these species are involved in SIDD, neurons were treated with either L-NOARG (1 mM) to block NO synthesis or PEG-SOD (100 U) to degrade superoxide. As shown in Fig. 3, stretched cells treated with L-NOARG were no longer significantly depolarized compared with controls (−54.9 ± 2.3 mV; n = 10 vs. −60.2 ± 0.8 mV; n = 9), although they remained somewhat depolarized. This suggests that L-NOARG may have been modestly protective. Stretched cells treated with PEG-SOD, however, remained significantly depolarized compared to controls (−53.7 ± 2.1 mV; n = 9 vs. −63.4 ± 1.9 mV; n = 8, P < 0.01). These results suggest that NO may contribute weakly to SIDD, whereas superoxide anion is unlikely to be involved in SIDD.

**Delayed depolarization of neuronal RMP by exogenous glutamate application**

Because SIDD is blocked by glutamate receptor antagonists (Tavalin et al. 1995), it was of interest to determine whether application of exogenous glutamate to neurons in the absence of stretch could mimic SIDD. Thus cortical neurons were exposed to 10 μM glutamate for 3 min at room temperature. This dose of glutamate was used because it has been reported to be sublethal (Choi et al. 1987; Coulter et al. 1992; Wang et al. 1994) and SIDD is also not associated with cell death (Tavalin et al. 1995). Neurons exposed to glutamate and then incubated for 1 h were found to be depolarized significantly compared with control neurons (−54.7 ± 2.7 mV; n = 7 vs. −61.7 ± 1.0 mV; n = 7, P < 0.05; Fig. 4). In addition, the input resistance of glutamate-treated neurons was not significantly different from controls (559 ± 70 MΩ; n = 7 vs. 603 ± 63 MΩ; n = 7), as is also the case for SIDD. Additionally, neurons that remained at room temperature after glutamate exposure were not depolarized (−62.0 ± 1.1 mV; n = 6; Fig. 4), suggesting that

![FIG. 2. Effect of inclusion of 5 mM ATP within internal pipette solution on SIDD. A: resting membrane potentials (RMPs) of control and stretched neurons were no longer significantly different when ATP was included in pipette (compare with Fig. 1A). B: ouabain depolarized control and stretched neurons to same extent when ATP was in pipette solution, suggesting that ATP relieved inhibition of Na pump seen in stretched cells dialyzed without ATP (compare with Fig. 1B). C: I-V relationship of control neurons was not affected by whole cell dialysis with pipette solution containing 5 mM ATP (●). I-V obtained in ouabain (○) shows that ouabain induced an inward current without alteration in slope conductance suggesting that Na pump current was not modified by presence of high ATP in pipette solution (compare with Fig. 1C). D: I-V relationship of a representative stretched cell dialyzed with 5 mM ATP (●). Zero current potential was similar to that of control cells. Enhanced sensitivity to ouabain (○) shows that ATP restored an inward pump current that was absent in stretched cells without pipette ATP (compare with Fig. 1D). These results suggest that inclusion of ATP within the patch pipette restored Na pump current and that Na pump current underlies SIDD.

![FIG. 3. Contribution of nitric oxide (NO) and superoxide to SIDD. Pretreatment with NO synthase inhibitor Nω-nitro-L-arginine (L-NOARG; 1 mM) was used to test whether NO production was involved in induction of SIDD. SIDD was attenuated partially, suggesting that NO may contribute to SIDD. Pretreatment with a polyethylene glycol conjugate of superoxide dismutase (PEG-SOD; 100 U) was used to assess involvement of superoxide in SIDD. Treatment with PEG-SOD did not offer any protection, suggesting that superoxide is unlikely to be involved in SIDD. *P < 0.01.](http://jn.physiology.org/article/10.1152/jn.1997.278.11.416)
incubation is required for this glutamate-induced persistent depolarization much like incubation is required for SIDD (Tavalin et al. 1995). These results suggest that glutamate produced a delayed depolarization by a mechanism similar to SIDD. This was further tested by applying ouabain to glutamate-treated neurons. Ouabain depolarized glutamate-treated neurons by $1.7 \pm 0.9$ mV ($n = 3$) similar to the blunted ouabain depolarization seen with stretched cells (Fig. 1A). These results suggest that glutamate-induced delayed depolarization, like SIDD, was mediated by Na pump inhibition.

**DISCUSSION**

The present study used an in vitro model of TBI (Ellis et al. 1995) to understand the mechanism mediating SIDD, a delayed electrical response to stretch injury we reported recently (Tavalin et al. 1995). Whole cell patch-clamp recordings indicated that the Na pump was inhibited in stretched neurons. This inhibition was likely due to reduced cellular energy levels because the RMPs of stretched neurons were depolarized when ATP was not included in the pipette solution but were restored to control levels by 5 mM ATP. Restoration of control RMPs in stretched neurons by ATP occurred in parallel with restoration of Na pump current, suggesting that Na pump inhibition underlies SIDD. This is consistent with our previous observation that SIDD was not associated with altered membrane conductance (Tavalin et al. 1995).

Control recordings made with no ATP in the pipette had intact Na pump current, which may seem paradoxical. One possible explanation for this is incomplete whole cell dialysis. However, RMPs were generally stable within a few minutes of establishing the whole-cell configuration and remained so for 30–45 min, suggesting that dialysis was not time limited. It may be more difficult to wash out substances, such as ATP, which are produced actively by cellular metabolism and tightly regulated. Thus our finding that supplying stretched neurons with ATP restores control RMPs and Na pump current suggests that SIDD is likely to reflect a metabolic disturbance that occurs after stretch injury.

Although we found that supplying exogenous ATP reduced SIDD, we can only infer that cellular ATP concentration is decreased. Increased ATP hydrolysis might occur due to Na pump stimulation by glutamate receptor activation (Fukuda and Prince 1992) and neuronal firing (Thomas 1972), both of which are required for SIDD (Tavalin et al. 1995). However, it is possible that intracellular ATP concentrations remained unchanged after stretch but that ADP concentration increased. Although increased intracellular ADP would reduce the ATP/ADP ratio and thus pump activity, other actions of ATP can not be excluded. However, we believe our metabolic hypothesis is reasonable given what is known about SIDD and previously reported effects of glutamate on neurons. Because SIDD requires calcium for its induction (Tavalin et al. 1995), calcium-dependent uncoupling of mitochondrial oxidative phosphorylation, which may be involved in excitotoxicity, also might contribute to SIDD. Because a large fraction of mitochondrial respiration fuels the Na pump even at rest (Astrup et al. 1981), mitochondrial disruption thus would lead to Na pump inhibition. Mitochondria play an important role in buffering glutamate-induced calcium entry (White and Reynolds 1995), and thus excessive calcium loads would likely disrupt oxidative phosphorylation. For example, the glutamate receptor agonist kainate has been reported to produce a calcium-dependent depolarization of mitochondrial membrane potential, which is known to compromise ATP synthesis (Bindokas and Miller 1995). Acidification of hippocampal neurons after glutamate exposure also has been attributed to calcium-dependent uncoupling of mitochondrial respiration (Wang et al. 1994). Although the specific mechanisms involved in reducing cellular energy levels with regards to SIDD remain unknown, the hypothesis that altered cellular metabolism underlies SIDD is consistent with reduced bioenergetic status previously linked to TBI through decreases in the ratio of phosphocreatine to inorganic phosphate (Faden et al. 1989) and excitotoxicity via decreases in cellular ATP levels (Rezt and Coyle 1982; Rothman et al. 1987). Additionally, the time course of SIDD (Tavalin et al. 1995) strongly resembles that for changes in energy charge following exposure of cerebellar granule cells to glutamate (Ankarcrona et al. 1995).

The glutamate- and calcium-dependent production of NO via stimulation of nitric oxide synthase has been suggested to contribute to excitotoxic neuronal damage (Dawson et al. 1992). We found that interfering with NO production appeared to attenuate SIDD but did not fully prevent its induction. How NO production may contribute to SIDD, however, remains unclear. Although it has been reported that NO inhibits mitochondrial electron transport (Dawson et al. 1992), which would be expected to decrease ATP synthesis, inhibition of NO synthesis also has been reported to enhance the Na pump activity in central neurons (Nathanson et al. 1995). Because the Na pump is important in terminating glutamate-induced depolarizations (Fukuda and Prince 1992), enhancement of Na pump activity may more
effectively terminate glutamate action after stretch and enhance neuronal repolarization.

Several lines of evidence suggest that reactive oxygen species (ROS) are produced after glutamate receptor activation and contribute to excitotoxicity (Coyle and Puttfarcken 1993). To test whether superoxide contributes to SIDD, PEG-SOD was used to enhance superoxide degradation. Although PEG-SOD did not prevent SIDD, we cannot exclude the possibility that other ROS contribute. The lack of an involvement of superoxide, however, may be consistent with our previous observation that SIDD is not associated with cell death (Tavalin et al. 1995).

Because glutamate receptor antagonists prevent SIDD (Tavalin et al. 1995) and glutamate levels are elevated after TBI (Faden et al. 1989; Katayama et al. 1990; Nilsson et al. 1990), it was important to determine whether glutamate alone could mimic SIDD in the absence of mechanical stretch. It is known that toxic glutamate exposure produces terminal neuronal depolarization and disruption of input resistance (Coulter et al. 1992). However, the glutamate treatment used in our present study was much more moderate in terms of concentration and duration of exposure than that which is used to cause cell death (Choi et al. 1987; Coulter 1992). Neurons exposed to our glutamate protocol without a subsequent incubation period had normal RMPs, consistent with the observation that neuronal RMPs return to baseline values after sublethal glutamate exposures (Coulter et al. 1992). However, our finding that incubation is required for glutamate to mimic SIDD may be related to the observation that sustained calcium entry or repeated glutamate exposures at 37°C inhibit the Na pump of hippocampal CA1 neurons (Fukuda and Prince 1992). Additionally, the time course of Na pump inhibition by calcium entry or glutamate exposures (Fukuda and Prince 1992) resembled the time course for SIDD induction (Tavalin et al. 1995). Our finding that glutamate inhibits the Na pump in a delayed manner thus appears consistent with these earlier studies. Our results further suggest that Na pump suppression after sustained calcium entry (Fukuda and Prince 1992) also may be due to reduced cellular energy levels. Although the results of the glutamate exposure experiments indicate that glutamate exposure alone is sufficient to mimic SIDD, mechanically induced alterations in glutamate receptors also may contribute to SIDD (Zhang et al. 1996).

We do not know why maintaining cells at room temperature after stretch-injury or glutamate exposure protected against SIDD. However, it is known that glutamate release from cortical neurons after NMDA or glutamate stimulation is attenuated by cooling, which may be a factor (Bruno et al. 1994). We favor the possibility, however, that prolonged incubation (≥15–60 min) at 37°C allows a [Ca2+]2-dependent intracellular cascade to occur that starts with the activation of NMDA receptors and involves a mitochondria-mediated alteration in ATP/ADP with the concomitant inhibition of the electrogenic Na pump. It seems reasonable to us that such a biochemical cascade might contain at least one temperature-dependent step.

The conditions employed in the present study must be considered in terms of extrapolating these findings to studies of adult neurons at physiological temperatures. Thus Na pump activity and pump current are known to increase with temperature (den Hertog and Ritchie 1969; Thompson and Prince 1986) and with development (Fukuda and Prince 1992; Haglund et al. 1985). This suggests that SIDD amplitude, which is in part a function of pump current amplitude, would be enhanced in older neurons and at warmer temperatures. However, neuronal RMP has been reported to be relatively unaltered by changes in temperature (Thompson et al. 1985) or postnatal developmental stage (McCormick and Prince 1987), whereas membrane input resistance decreases with increased temperature (Thompson et al. 1985) and postnatal age (Fukuda and Prince 1992; McCormick and Prince). These findings suggest that the electrogenic Na pump contribution to RMP and consequently SIDD actually may be similar at various temperatures or at different stages of postnatal development.

Na pump inhibition, as well as reductions in energy levels due to metabolic poisoning, oxygen or glucose deprivation, enhance glutamate neurotoxicity (Brines and Robbins 1992; Brines et al. 1995; Novelli et al. 1988). Our linkage of SIDD to Na pump inhibition might explain why sublethal TBI, which is associated with increased glutamate, increases neuronal vulnerability to delayed secondary insults like ischemia (Jenkins et al. 1989). Additionally, the Na pump plays an important role in restoration of ionic gradients and cell repolarization after tetanic stimulation (Thomas 1972) or brief glutamate applications (Fukuda and Prince 1992), pump inhibition may alter membrane excitability and neuronal information processing. Such alterations could contribute to the behavioral deficits that occur in the wake of TBI.

In conclusion, we report that Na pump inhibition underlies SIDD and appears to be secondary to a possible metabolic derangement. The overall contribution of NO to SIDD appears to be small. Superoxide anion, a potential distal effector of glutamate action, does not appear to be involved at the degree of injury used in this study. Additionally, exogenous glutamate application alone was sufficient to mimic SIDD, consistent with the hypothesis that similar mechanisms may contribute to SIDD and excitotoxicity. Na pump inhibition due glutamate receptor activation therefore appears to be a major mechanism underlying SIDD. Thus decreased Na pump activity via altered energy metabolism may contribute to the pathophysiology of TBI as has been suggested for neurodegenerative diseases (Beal et al. 1993; Lees 1991).

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REFERENCES


Astrup, J., Sorensen, P. M., and Sorensen, H. R. Oxygen and glucose...