Pharmacological Inhibition of the Na⁺/Ca²⁺ Exchanger Enhances Depolarizations Induced by Oxygen/Glucose Deprivation but Not Responses to Excitatory Amino Acids in Rat Striatal Neurons

Paolo Calabresi, MD; Girolama A. Marfia, MD; Salvatore Amoroso, MD; Antonio Pisani, MD; Giorgio Bernardi, MD

Background and Purpose—Neuronal Na⁺/Ca²⁺ exchanger plays a relevant role in maintaining intracellular Ca²⁺ and Na⁺ levels under physiological and pathological conditions. However, the role of this exchanger in excitotoxicity and ischemia-induced neuronal injury is still controversial and has never been studied in the same neuronal subtypes.

Methods—We investigated the effects of bepridil and 3′,4′-dichlorobenzamil (DCB), 2 blockers of the Na⁺/Ca²⁺ exchanger, in rat striatal spiny neurons by utilizing intracellular recordings in brain slice preparations to compare the action of these drugs on the membrane potential changes induced either by oxygen and glucose deprivation (OGD) or by excitatory amino acids (EAAs).

Results—Bepridil (3 to 100 μmol/L) and DCB (3 to 100 μmol/L) caused a dose-dependent enhancement of the OGD-induced depolarization measured in striatal neurons. The EC₅₀ values for these effects were 31 μmol/L and 29 μmol/L, respectively. At these concentrations neither bepridil nor DCB altered the resting membrane properties of the recorded cells (membrane potential, input resistance, and current-voltage relationship). The effects of bepridil and DCB on the OGD-induced membrane depolarization persisted in the presence of d-2-amino-5-phosphonovalerate (50 μmol/L) plus 6-cyano-7-nitroquinoxaline-2,3-dione (20 μmol/L), which suggests that they were not mediated by an enhanced release of EAAs. Neither tetrodotoxin (1 μmol/L) nor nifedipine (10 μmol/L) affect the actions of these 2 blockers of the Na⁺/Ca²⁺ exchanger, which indicates that voltage-dependent Na⁺ channels and t-type Ca²⁺ channels were not involved in the enhancement of the OGD-induced depolarization. Conversely, the OGD-induced membrane depolarization was not altered by 5-[(N,N-hexamethylene) amiloride (1 to 3 μmol/L), an inhibitor of the Na⁺/H⁺ exchanger, which suggests that this antiporter did not play a prominent role in the OGD-induced membrane depolarization recorded from striatal neurons. Bepridil (3 to 100 μmol/L) and DCB (3 to 100 μmol/L) did not modify the amplitude of the excitatory postsynaptic potentials evoked by cortical stimulation. Moreover, these blockers did not affect membrane depolarizations caused by brief applications of glutamate (0.3 to 1 mmol/L), AMPA (0.3 to 1 μmol/L), and NMDA (10 to 30 μmol/L).

Conclusions—These results provide pharmacological evidence that the activation of the Na⁺/Ca²⁺ exchanger exerts a protective role during the early phase of OGD in striatal neurons, although it does not shape the amplitude and the duration of the electrophysiological responses of these cells to EAA. (Stroke. 1999;30:1687-1693.)

Key Words: bepridil 3,4-dichlorobenzamil excitatory amino acids ischemia sodium-hydrogen antiporter synaptic transmission rats

An important event that occurs during ischemia is the loss of ionic homeostasis.¹ A long-lasting disruption of ionic gradients causes permanent cell injury. Accordingly, a major hypothesis concerning the mechanisms underlying neuronal injury after energy deprivation postulates that an increase in intracellular Ca²⁺ represents the critical event to trigger the cascade of processes leading to cell death.² Various experimental findings have also stressed the important role of Na⁺.

See Editorial Comment, page 1694

in the generation of cell damage induced by energy deprivation. Hypoxia-induced membrane depolarization is markedly attenuated by the removal of extracellular Na⁺ in various central neurons.³,⁴ Moreover, it has been reported that intracellular Na⁺ levels increase during anoxia and ischemia.⁵,⁶ A potential source of both Ca²⁺ and Na⁺ rise during ischemia is

Received February 8, 1999; final revision received April 14, 1999; accepted May 3, 1999.
From Clinica Neurologica, Dipartimento di Neuroscienze, Università Tor Vergata (P.C., G.A.M., A.P., G.B.), and IRCCS Ospedale Santa Lucia (P.C., G.B.), Rome, and Dipartimento di Neuroscienze, Università Federico II (S.A.); Naples, Italy.
Correspondence to Paolo Calabresi, Clinica Neurologica, Dipartimento di Neuroscienze, Università “Tor Vergata,” via di Tor Vergata 135, 00133, Rome, Italy. E-mail calabre@uniroma2.it
© 1999 American Heart Association, Inc.
Stroke is available at http://www.strokeaha.org

1687
represented by the activation of ionotropic glutamate receptors, because an increased release of glutamate has been observed during both in vitro anoxia and in vivo ischemia. Neuronal Na\(^+/\)Ca\(^{2+}\) exchanger may also play an important role in maintaining intracellular Ca\(^{2+}\) and Na\(^+\) levels in normal and pathological states. Under physiological conditions, Na\(^+/\)Ca\(^{2+}\) exchanger transports Na\(^+\) in and Ca\(^{2+}\) out (forward mode). Membrane depolarizations induced either by ischemia or by excessive activation of excitatory amino acid (EAA) receptors may favor the reverse operation of the exchanger, moving Ca\(^{2+}\) in and Na\(^+\) out (reverse mode). The role of the Na\(^+/\)Ca\(^{2+}\) exchanger in excitotoxicity and ischemia-induced neuronal injury is, however, still controversial. It has been reported that Na\(^+/\)Ca\(^{2+}\) exchanger blockers are effective in protection against anoxic damage in the white matter. Conversely, Amoroso et al demonstrated that the blockade of the Na\(^+/\)Ca\(^{2+}\) exchanger enhanced hypoxia-induced LDH release in glioma cells, providing pharmacological evidence that the activation of the Na\(^+/\)Ca\(^{2+}\) exchanger plays a protective role during energy failure. Glutamate-induced increase in intracellular Na\(^+\) may also stimulate the reverse operation of the exchanger, increasing intracellular Ca\(^{2+}\) overload and potentiating injury. On the other hand, it has been shown that inhibition of the Na\(^+/\)Ca\(^{2+}\) exchanger enhances delayed neuronal death elicited by glutamate in cerebellar granule cells cultures, which suggests a neuroprotective function of this exchanger during excitotoxicity.

In this work, therefore, we sought to further elucidate the possible modulatory role of Na\(^+/\)Ca\(^{2+}\) exchanger during combined oxygen and glucose deprivation (OGD) and during the action of EAAs in striatal spiny neurons, a neuronal subtype that is particularly vulnerable both to energy deprivation and excitotoxicity. For this reason, we have tested the effects of bepridil and DCB, inhibitors of this exchanger, on the membrane depolarization induced by OGD in striatal spiny neurons intracellularly recorded from a brain slice preparation. Thus, we have compared these effects with those observed after blockade of Na\(^+/\)Ca\(^{2+}\) exchanger during membrane depolarizations caused either by endogenous glutamate or by exogenous applications of EAA agonists.

**Materials and Methods**

**Preparation and Maintenance of the Slices**

Male Wistar rats (Morini, Italy; 150 to 250 g) were used. Preparation and maintenance of coronal slices have been described previously and followed the institutional guidelines. Briefly, animals were anesthetized with ether and killed by cervical dislocation; corticostriatal coronal slices (200 to 300 μm) were then prepared from tissue blocks of the brain with the use of a Vibratome. A single slice was transferred to a recording chamber and submerged in a continuously flowing Krebs solution (35°C, 2 to 3 mL/min) gassed with 95% O\(_2\)–5% CO\(_2\). To study OGD in striatal neurons, slices were deprived of glucose by removing glucose totally from the perfusate and by adding sucrose to balance the osmolarity. This solution was gassed with a mixture of 95% N\(_2\)–5% CO\(_2\) instead of the normal gas mixture. In some experiments the osmolarity was balanced by increasing the NaCl concentration. Because experiments performed by use of these different procedures to replace glucose gave similar results, all the data were pooled together.

Ischemic solutions entered the recording chamber no later than 20 seconds after turning a 3-way tap.

**Recording Technique**

In most of the experiments the intracellular recording electrodes were filled with 2 mol/L KCl (30 to 60 MΩ). An Axoclamp 2A amplifier (Axon Instruments) was used for intracellular recordings. Traces were displayed on an oscilloscope and stored on a digital system. For synaptic stimulation, bipolar electrodes were used. These stimulating electrodes were located either in the cortical areas close to the recording electrode or in the white matter between the cortex and the striatum to activate corticostriatal fibers. In some experiments biocytin (Sigma) was used in the intracellular electrode to stain the neurons. In these cases, biocytin at concentrations of 2% to 4% was added to a 0.5 mol/L-KCl pipette solution. Slices containing neurons stained with biocytin were fixed in paraformaldehyde (in 0.1 mol/L phosphate buffer at pH 7.4) overnight and processed according to published protocols.

**Data Analysis and Drug Applications**

Quantitative data on changes of membrane depolarization were usually expressed as a percentage of the control value. Values given in the text and in the figures are mean ± SEM of changes in the respective cell populations. The Student t test (for paired and unpaired observations) was used to compare the means. The characteristics of action potentials and of current-voltage curves in different experimental conditions were studied with use of a fast chart recorder and a digital system (Nicolet System 400: Benchtop Waveform Acquisition System, Sekonic). To calculate the area of the depolarization caused either by the application of the ischemic medium or by the application of EAAs, data were displayed on a high-gain strip-chart recorder (Gould RS 3400) and the area was calculated by the use of a digital planimeter. Responses that did not show any tendency to recovery after the offset of OGD or EAA application were discarded from the statistics. Drugs were administered by dissolving them to the desired final concentration in the saline and by switching the perfusion from control saline to drug-containing saline. 6-Cyano-7-nitroquinolinoxaline-2,3-dione (CNQX) was from Tocris, D-2-Amino-5-phosphonovalerate (D-APV), bepirdil, and tetrodotoxin (TTX) were from Sigma. Nifedipine was from RBI, DCB and 5-(N,N-hexamethylene) amiloride were from E.J. Crisgoe, Jr (Nacogdoches, Tex).

**Results**

**Electrophysiological Properties of the Recorded Neurons**

Conventional sharp-microelectrode intracellular recordings were obtained from 81 electrophysiologically identified “principal” spiny cells. The main characteristics of these cells have been described in detail previously both in vivo and in vitro. These cells had high resting membrane potential (−84 ± 5 mV), relatively low apparent input resistance (38 ± 8 MΩ) when measured at the resting potentials from the amplitude of small (<10 mV) hyperpolarizing electrotonic pulses, action potentials of short duration (1.1 ± 0.3 ms), and high amplitude (102 ± 4 mV). They were silent at rest and showed membrane rectification and tonic firing activity during depolarizing current pulses. In 22 of the 81 recorded spiny neurons, the electrophysiological identification was confirmed by a morphological analysis obtained by use of biocytin staining (data not shown).

**Effect of Bepridil and DCB on the OGD-Induced Membrane Depolarization**

OGD induced a membrane depolarization in all the recorded cells. The amplitude and the duration of the OGD-induced
membrane depolarization was dependent on the time of exposure to the ischemic medium. As we have previously reported for hypoxia and aglycemia, this electrophysiological event was associated to a decreased input resistance (n=10, data not shown). Moreover, we found that the time course of the ischemic depolarization was much faster than those observed for hypoxia and aglycemia alone. In fact, a brief period (90 seconds) of OGD produced a significant membrane depolarization in the large majority of the recorded neurons (65 of 70 cells) whereas similar periods of aglycemia and hypoxia alone induced either no effect or much smaller changes of the membrane potential. In the remaining 5 striatal neurons, longer periods (2 to 3 minutes) of OGD were required to produce the membrane depolarization. To study the possible modulatory role of the Na⁺/Ca²⁺ exchanger on the ischemic depolarization, we applied bepridil, a blocker of this exchanger, 5 to 10 minutes before the brief ischemic exposure. In all the experiments using either bepridil or other pharmacological blockers, at least 2 control ischemic responses were obtained. Only neurons showing constant control responses to OGD were considered for further pharmacological analysis. Bepridil enhanced in a dose-dependent manner (10 to 100 μmol/L, n=30) the OGD-induced membrane depolarization with an EC₅₀ of 31 μmol/L (Figure 1). We also tested whether DCB, a more specific blocker of the Na⁺/Ca²⁺ exchanger, could mimic the electrophysiological effect obtained with bepridil. As shown in Figure 2, DCB also increased in a dose-dependent manner (10 to 100 μmol/L, n=25) the ischemic depolarization with an EC₅₀ of 29 μmol/L. We also investigated the possibility that bepridil and DCB altered the resting membrane properties of the recorded cells. At the doses used in the present study, neither bepridil (n=30) nor DCB (n=25) altered resting membrane potential, input resistance, and current-voltage relationship in the subthreshold range (Figure 3).

### Effects of Bepridil and DCB Are Not Affected by Antagonists of Excitatory Amino Acids, TTX, and Ca²⁺ Channel Blockers

It has been reported that bepridil might produce effects other than the blockade of the Na⁺/Ca²⁺ exchanger. In particular, it has recently been shown that bepridil modulates NMDA currents in rat hippocampal neurons. Moreover, bepridil is able to enhance the release of EAs induced by energy deprivation in hippocampal slices. For this reason, it was crucial to test (1) the possible involvement of an increased release of EAs in the facilitatory effects of bepridil and DCB on the OGD-induced membrane depolarization and (2) the possibility that the pharmacological effects of these blockers were due to a postsynaptic modulation of neuronal responses to EAs released during OGD. To address these issues, we incubated the slices in the presence of 50 μmol/L APV plus 20 μmol/L CNQX, which antagonize NMDA- and AMPA-like glutamate receptors, respectively. This pharmacological treatment affected neither the membrane depolarization induced by OGD (n=7, data not shown) nor the facilitatory action of bepridil (n=4, Figures 4A and 5) and DCB (n=4, Figure 5). Bepridil may also have a direct effect on voltage-dependent Na⁺ channels. Thus, we investigated whether TTX, a Na⁺ channel blocker, affected the effects of bepridil and DCB. TTX did not alter the OGD-induced membrane depolarization (n=3, data not shown) or the pharmacological effects on OGD produced by bepridil (n=4, Figures 4B and 5) and DCB (n=4, Figure 5). Furthermore, because it has been reported that bepridil modulates L-type Ca²⁺ channels, we studied the pharmacological action of bepridil and DCB in the presence of 10 μmol/L nifedipine, a blocker of L-type Ca²⁺ channels. Also, this channel blocker failed to affect the OGD-induced depolarization (n=3, data not shown) and the actions of bepridil (n=4, Figures 4C and 5) and DCB (n=4, Figure 5).

### Lack of Effect of Bepridil and DCB on Responses to EAs

To further investigate the possible interaction between the activity of the Na⁺/Ca²⁺ exchanger and the electrophysiological effects of EAs on striatal neurons both at presynaptic...
and postsynaptic sites, we studied the effect of bepridil and DCB in various experimental conditions. First, we measured the effect of these blockers on the amplitude of EPSPs evoked by cortical stimulation. Neither bepridil (30 μmol/L, Figures 6A and E) nor DCB (30 μmol/L, data not shown) affected the amplitude of these synaptic potentials. Then, we tested a possible action of these 2 drugs on the membrane depolarizations induced by brief applications of glutamate (1 mmol/L, 10 to 20 seconds), AMPA (0.3 to 1 μmol/L, 30 to 60 seconds), and NMDA (10 to 30 μmol/L, 30 to 60 seconds). Neither bepridil (30 μmol/L, Figures 6B, C, D, and E) nor DCB (30 μmol/L, data not shown) were able to modify these membrane responses.

**Lack of Effect of HMA on the OGD-Induced Membrane Depolarization**

It has been suggested that intracellular acidification which occurs during ischemia activates the Na⁺/H⁺ exchanger, resulting in Na⁺ loading. Pharmacological inhibition of the Na⁺/H⁺ exchanger has been reported to inhibit the rise in intracellular Na⁺ and to delay the rise in Ca²⁺ in cardiac myocytes. To evaluate the possible involvement of the Na⁺/H⁺ exchanger in our experimental model of in vitro ischemia, we investigated the effects of HMA, a blocker of this exchanger, on the OGD-induced membrane depolarization. HMA (1 to 3 μmol/L) did not affect this electrophysiological event in any of the tested neurons. In fact, the OGD-induced depolarization was not significantly altered by HMA (102±5%, n=10, P>0.05) (data not shown).

**Discussion**

The main finding of our study is that the pharmacological inhibition of the Na⁺/Ca²⁺ exchanger enhances the OGD-induced membrane depolarization in striatal spiny neurons whereas it does not affect glutamate-mediated synaptic potentials and membrane responses to EAAs. Although the pharmacological data supporting this observation appear highly consistent, the interpretation of the mechanism involved in this facilitatory action is far from being obvious and requires an accurate consideration of the ionic mechanisms underlying the ischemic depolarization in striatal spiny neurons. We recently found that the OGD-induced membrane depolarization is associated with a rise in intracellular concentrations of both Na⁺ and
Ca²⁺. However, the buffering of intracellular Ca²⁺ by injecting BAPTA, a Ca²⁺ chelator, via the recording pipette did not reduce the ischemic depolarization. On the contrary, the lowering of the extracellular Na⁺ concentration significantly reduced this event. Thus, we argued that Na⁺ influx plays a major role in the generation of this early electrical event. The source of this Na⁺ influx was not identified. In fact, neither the blockade of voltage-dependent Na⁺ channels by TTX nor the antagonism of the ionotropic glutamate receptors by CNQX and APV significantly altered the ischemic depolarization, which suggests that a TTX insensitive, nonselective cation conductance might play a role in this event.29

It has been shown that the Na⁺/Ca²⁺ exchanger becomes reversed during hypoxia,12,19 and there is a growing amount of literature on the role of this exchanger during energy deprivation in a variety of CNS tissue.7,32 Blockers of the Na⁺/Ca²⁺ exchanger afforded protection from anoxic injury in the rat optic nerve preparation.12 This protection was attributed, however, to a net reduction in intracellular Ca²⁺ due to the blockade of this exchanger. The authors argued that the anoxia-induced increase in intracellular Na⁺ concentration acts to reverse the driving force for the Na⁺/Ca²⁺ exchanger, thereby causing an increase in intracellular Ca²⁺. This increase caused neuronal injury and was inhibited by the blockade of the Na⁺/Ca²⁺ exchanger.

From our previous and current experiments, we propose a different interpretation of the role of this exchanger during OGD in spiny neurons. We suggest that the activation of the Na⁺/Ca²⁺ exchanger during the ischemic depolarization has a protective role, because we found that bepridil and DCB enhanced this electrophysiological event. Thus, we postulate that during OGD in striatal spiny neurons, as well as in optic nerve preparation, the rise in intracellular Na⁺ levels might force this exchanger to operate in a reverse mode (as a Na⁺ extruding system). However, in striatal spiny neurons, unlike in optic nerve preparation, the critical event for the OGD-induced injury is represented by the rise of intracellular Na⁺ rather than the intracellular increase of Ca²⁺. This conclusion contrasts with results from previous studies using other experimental models.33 In agreement with our findings, it has been recently reported that activation of the Na⁺/Ca²⁺ exchanger protects C6 glioma cells during chemical hypoxia.13 However, future studies in brain slices utilizing ion measurements of intracellular Ca²⁺ and Na⁺ are required to confirm this hypothesis. Unfortunately, at present, most of the drugs interfering with the activity of Na⁺/Ca²⁺ exchanger and Na⁺/H⁺ antiporter are amiloride derivatives, and their use is

Figure 4. The pharmacological action of bepridil is not affected by the blockade of ionotropic glutamate receptors, of voltage-dependent Na⁺ channels, or of L-type Ca²⁺ channels. A, The incubation of the slice in the presence of 50 μmol/L APV plus 20 μmol/L CNQX did not block the bepridil-induced increase of the ischemic depolarization recorded from a spiny neuron (a, control; b, 30 μmol/L bepridil) (RMP = −86 mV). B, The incubation of the slice in the presence of 1 μmol/L TTX did not block the bepridil-induced increase of the ischemic depolarization recorded from a spiny neuron (a, control; b, 30 μmol/L bepridil) (RMP = −88 mV). C, The incubation of the slice in the presence of 10 μmol/L nifedipine did not block the bepridil-induced increase of the ischemic depolarization recorded from a spiny neuron (a, control; b, 30 μmol/L bepridil) (RMP = −87 mV). Calibrations reported in C apply also to A and B.

Figure 5. Lack of effect of various experimental conditions to block the pharmacological actions of bepridil and DCB. The graph shows that the increase of the area of the OGD-induced membrane depolarization produced either by bepridil or by DCB is not altered the blockade of ionotropic glutamate receptors (50 μmol/L APV plus 20 μmol/L CNQX), of voltage-dependent Na⁺ channels (1 μmol/L TTX), or of L-type Ca²⁺ channels (10 μmol/L nifedipine).
Bepridil does not affect corticostriatal EPSPs and membrane depolarizations induced by brief applications of either glutamate or agonists of ionotropic glutamate receptors. A, The traces show that corticostriatal EPSP recorded from a striatal spiny neuron (a) was not affected by 30 μmol/L bepridil (b; RMP = −84 mV). B, Brief applications of exogenous glutamate (1 mmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −86 mV). C, Brief applications of AMPA (1 μmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −87 mV). D, Brief applications of NMDA (30 μmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −85 mV). Calibration reported in B apply also to C and D. The graph shows that the EPSP amplitude and the membrane depolarizations induced by glutamate, AMPA, and NMDA were not affected by 30 μmol/L bepridil.

Bepridil does not affect corticostriatal EPSPs and membrane depolarizations induced by brief applications of either glutamate or agonists of ionotropic glutamate receptors. A, The traces show that corticostriatal EPSP recorded from a striatal spiny neuron (a) was not affected by 30 μmol/L bepridil (b; RMP = −84 mV). B, Brief applications of exogenous glutamate (1 mmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −86 mV). C, Brief applications of AMPA (1 μmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −87 mV). D, Brief applications of NMDA (30 μmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −85 mV). Calibration reported in B apply also to C and D. The graph shows that the EPSP amplitude and the membrane depolarizations induced by glutamate, AMPA, and NMDA were not affected by 30 μmol/L bepridil.

Bepridil does not affect corticostriatal EPSPs and membrane depolarizations induced by brief applications of either glutamate or agonists of ionotropic glutamate receptors. A, The traces show that corticostriatal EPSP recorded from a striatal spiny neuron (a) was not affected by 30 μmol/L bepridil (b; RMP = −84 mV). B, Brief applications of exogenous glutamate (1 mmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −86 mV). C, Brief applications of AMPA (1 μmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −87 mV). D, Brief applications of NMDA (30 μmol/L) induced a membrane depolarization (a) that is not affected by 30 μmol/L bepridil (b; RMP = −85 mV). Calibration reported in B apply also to C and D. The graph shows that the EPSP amplitude and the membrane depolarizations induced by glutamate, AMPA, and NMDA were not affected by 30 μmol/L bepridil.
extruding most of the excess of Ca\(^{2+}\) admitted during the activation of ionotropic glutamate receptors. Moreover, the Na\(^+/K^+\) ATP-dependent pump is able to provide an effective mechanism to limit excessive intracellular Na\(^+\) overload during the action of various EAs.\(^8\) The activity of these 2 protective mechanisms is impaired during energy deprivation, because ATP production is dramatically reduced. Thus, in this physiopathological condition, the protective role of the Na\(^+/Ca^{2+}\) exchanger operating in its reverse mode is unmasked. Finally, we would like to close this study with 2 notes of caution in the interpretation of the presented data. First, in our experimental model we have analyzed membrane depolarizations induced by OGD. It is commonly assumed that these depolarizations may ultimately lead to neuronal death; however, a morphological analysis of the tissue alterations induced by OGD in our model has not been performed. Second, our conclusions stand on in vitro data; future studies utilizing in vivo models of ischemia would be of great interest to further clarify the role of Na\(^+/Ca^{2+}\) exchanger in this pathological event.

**Acknowledgments**

We wish to thank M. Tolu for the excellent technical assistance. This study was supported by a grant from Biomed Project to Dr Calabresi (BMH4-97-2215), a Telethon grant to Dr Calabresi (E. 729), and a MURST/CNR grant to Dr Bernardi (legge 95/95).

**References**

Increased intracellular calcium (Ca$^{2+}$) has been widely demonstrated to be a focal point in cell death cascades for many types of neurons and supporting cells. The relative significance of increased intracellular sodium supporting cells. The relative significance of increased intracellular sodium (Na$^+$) in neuronal survival after anoxic depolarization and energy failure has not been as well studied. If elevated intracellular Na$^+$ also contributes to neuronal demise, what are the sources of Na$^+$ influx and which extrusion mechanisms dominate in the strategy for control of transmembrane ionic gradients? When ATP production is persistently inadequate, energy dependent Na$^+$/K$^+$ pumps and Ca$^{2+}$ ATPase unlikely defenders of cation homeostasis. Therefore, the membrane Na$^+$/Ca$^{2+}$ exchanger, which in physiological forward mode transports Na$^+$ in and Ca$^{2+}$ out of the cell, may become instrumental in reducing anoxic, or exacerbating, damage in central neurons. Given that the exchanger can be forced into reverse mode, moving Ca$^{2+}$ into the neuron$^{1-3}$ and that exchange inhibitors can improve damage in several experimental paradigms and tissue types, it has been argued that the Na$^+$/Ca$^{2+}$ exchanger potentiates anoxic, hypoxic, and excitotoxic injury (for reviews, see References 4 and 5). This is the central and controversial issue addressed by the preceding paper. Using a well-established model of oxygen/glucose deprivation (OGD) in brain slices, the authors provide new information about the role of the Na$^+$/Ca$^{2+}$ exchanger in striatal spiny neurons, which are vulnerable to energy depletion and to glutamate excess. They clearly show that inhibitory agents which block the exchange enhance OGD-induced depolarization, suggesting a protective role. Moreover, the enhanced depolarization was specific to OGD and was not observed when neurons were challenged at ionotropic glutamate receptor activation in varying forms and doses. Interactions with other key regulators of Na$^+$ and Ca$^{2+}$ were reasonably excluded, including voltage-dependent Na$^+$ channels, L-type Ca$^{2+}$ channels, and the Na$^+$/H$^+$ antiporter. Therefore, the importance of Na$^+$/Ca$^{2+}$ exchange in energy failure is not trivial. It should be noted that the evidence provided is exclusively pharmacological and assumes reasonable specificity and efficacy of the antagonists employed. The latter point was not demonstrated; however, 2 different agents were used with quite similar results. From a clinical perspective, what remains to be shown is morphological evidence of protection in this neuronal subtype or that enhanced Na$^+$/Ca$^{2+}$ exchange is beneficial to injured or recovering cells.

Patricia D. Hurn, PhD, Guest Editor
Anesthesiology/Critical Care Medicine
Johns Hopkins Medical Institutions
Baltimore, Maryland

References
Pharmacological Inhibition of the Na⁺/Ca²⁺ Exchanger Enhances Depolarizations Induced by Oxygen/Glucose Deprivation but Not Responses to Excitatory Amino Acids in Rat Striatal Neurons

Paolo Calabresi, Girolama A. Marfia, Salvatore Amoroso, Antonio Pisani and Giorgio Bernardi

Stroke. 1999;30:1687-1694
doi: 10.1161/01.STR.30.8.1687

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/30/8/1687

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/