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Neurologic Preservation by Na^+-H^+ Exchange Inhibition Prior to 90 Minutes of Hypothermic Circulatory Arrest

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Background. The effects of pretreatment with cariporide (HOE 642 Aventis Pharma, Strasbourg - Cedex, France), a Na^+–H^+ exchanger (NHE) blocker, were studied in a cerebral ischemia–reperfusion model of hypothermic arrest.

Methods. Fifteen Yorkshire-Duroc pigs (37.1 ± 4.2 kg) underwent femoral–jugular bypass and 90 minutes of deep hypothermic circulatory arrest at 19°C. Ten animals were untreated, whereas 5 received 5 mg/kg of intravenous cariporide before cooling. After rewarming and off cardiopulmonary bypass, the pigs were weaned from anesthesia and followed for 24 hours. A standardized neurologic scoring system assessed brain functional recovery. Biochemical markers were used to analyze cellular injury. Control studies without circulatory arrest were done in 2 animals that underwent similar cooling and rewarming.

Results. Neurologic recovery was rapid and complete in the nonischemic controls and in all pretreated animals. Conversely, at 24 hours, all untreated pigs exhibited a cloudy or stuporous level of consciousness, abnormal positioning, and with only one exception, could not sit or stand. The gradation of neurologic score (evaluating central nervous system, motor and sensory functions, respiration condition, level of consciousness, and behavior) was 0 ± 0 (0 = normal, 500 = brain death) in the treated group, compared with 124 ± 59 in the untreated animals. Biochemical analysis showed every variable of whole-body injury (including conjugated dienes ($p < 0.05$), serum aspartate amino transferase ($p < 0.01$), creatine kinase $p < 0.001$) and endothelin-1 ($p < 0.001$) to be higher in the untreated group.

Conclusions. NHE function alters experimental brain ischemia–reperfusion damage. These observations imply that NHE inhibition therapy before ischemia may improve neurologic protection in adult and infant patients undergoing cerebral ischemia during procedures that use hypothermic circulatory arrest.

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The intracellular accumulation of calcium plays a major role in early and delayed neuronal death after brain ischemia and reperfusion [1]. Although calcium-mediated damage was initially studied for ischemia–reperfusion injury in the heart, clear-cut evidence in the last two decades [2] relates calcium overload to neuronal necrosis.

The final common pathway of calcium-related damage involves several mechanisms, including

- accumulation of glutamate and other neurotransmitters for adverse synaptic triggering of calcium entry [3],
- the opening of other adverse calcium channels through oxygen radical damage, especially with H_2O_2, [4],
- pathologic sequestration of cellular or phospholipid-bound calcium into the endoplasmic reticulum to cause uncontrolled release and predispose to raised calcium concentrations [5], or
- evolution of a reverse mode of the normal pathway of calcium extrusion that involves sodium–calcium exchange [6].

Our working hypothesis tested if the interruption of this Na^+–H^+ exchange (NHE) pathway affects brain ischemia–reperfusion damage.

The NHE mechanism is inactive during normal perfusion [7], but becomes the main dynamic buffering system of the cell during ischemia and extrudes protons for sodium in an electro-neutral exchange. The adenosine triphosphate (ATP)-dependent sodium–potassium exchanger that extrudes sodium to prevent calcium overload becomes impaired by prolonged ischemia and reperfusion. The ATP independent Na^+–Ca^{2+} exchanger persists in its efforts to reduce cellular sodium accumulation, so that subsequent calcium overload develops through its reversed activity. Secondary water accumulation follows sodium overload, resulting in edema that ultimately may impair blood flow and contributes to the leakage of excitatory amino acids [1].

The aims of this study were to test if (1) activity of the

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Table 1. Neurologic Deficit Scoring System[a]

(A) Central Nerve Function				
Variable	Side	Normal	Weak	Absent
Pupil size	R	0	2	5
	L	0	2	5
Light reflex	R	0	2	5
	L	0	2	5
Eye position	R	0	2	5
	L	0	2	5
Lid reflex	R	0	2	5
	L	0	2	5
Corneal reflex	R	0	2	5
	L	0	2	5
Ciliospinal reflex	R	0	2	5
	L	0	2	5
Oculocephalic reflex		0	5	10
Auditory reflex		0	5	10
Gag reflex		0	5	10
Carinal reflex		0	5	10

(B) Respiration	
Condition	
Normal	0
Hyperventilation	25
Abnormal	50
Apnea	100

(C) Motor and Sensory function			
Variable	Normal	Weak	Absent
Stretch reflex	0	10	25
Motor response to pain	0	10	25
Positioning	0	10	25
Muscle tonus	0	10	25

(D) Level of Consciousness	
Normal	0
Cloudy	30
Delirium	45
Stupor	60
Coma	100

(E) Behavior			
Variable	Present/ Normal	Weak	Absent
Drinking	0	···	15
Chewing	0	···	15
Sitting	0	···	15
Standing	0	···	15
Walking	0	20	40

[a] Total = sum of all sections (0 = normal; 500 = brain death).

NHE is a factor in neurologic damage in temporary brain ischemia, and (2) if NHE inhibition limits brain reperfusion injury by using pretreatment with cariporide (HOE 642 Aventis Pharma, Strasbourg - Cedex, France), a NHE blocker.

Material and Methods

All animals received humane care in compliance with the 1996 NRC Guide for the Care and Use of Laboratory Animals, available at http://www.nap.edu/readingroom/books/labrats/contents.html.

Seventeen Yorkshire-Duroc pigs (27 to 34.5 kg) were premedicated (ketamine 15 mg/kg and diazepam 0.5 mg/kg, intramuscularly) and anesthetized with inhaled isoflurane 1.5% (minimum alveolar concentration, 1%) throughout the operation. Support with a volume-controlled ventilator (Servo 900C, Siemens-Elema, Sweden) was started after endotracheal intubation. Sterile surgical technique was used in all animals. The mammary artery and vein were cannulated 2 cm after exiting the thoracic cavity, and arterial blood gases were measured to keep oxygen tension, carbon dioxide tension, and pH values within the normal range. A balloon-tipped pulmonary artery catheter (Model 132F5, Baxter Healthcare Corp, Irvine, CA) through the internal jugular vein measured cardiac output (thermodilution technique) and pulmonary artery pressure.

After systemic heparinization (300 U/kg), a 10F arterial cannula (Medtronic, Inc, Minneapolis, MN) was inserted in the superficial femoral artery and a 17F venous cannula (Medtronic, Inc) in the right atrium through the superficial jugular vein. Extracorporeal circulation with a membrane oxygenator (Affinity NT 541, Medtronic, Inc) and extracorporeal pump (Sarns, Ann Arbor, MI) included a circuit primed with 1000 mL of Plasma-Lyte solutions (Baxter Healthcare Corp., Irvine, CA), 700 mL stored porcine packed blood, and calcium chloride for normocalcemia (1.0 to 1.2 mmol/L).

Experimental Protocol

Cardiopulmonary bypass (CPB) was started at an oxygen tension of 300 mm Hg and an aortic pressure of 50 to 70 mm Hg, with flow adjusted to keep approximately 70% mixed venous oxygen saturation. To facilitate venous return, active suction by a centrifugal pump (Biopump BPX-80, Medtronic, Inc) was maintained during CPB. Animals were cooled to rectal temperature of 19°C by using a heat exchanger and a heating–cooling blanket. The pH was maintained at 7.40 by means of α-stat principles, with an arterial P_{CO_2} of 35 to 40 mm Hg, uncorrected for temperature. Hematocrit value was adjusted to 25% to 30% with donor blood and Plasma-Lyte, and calcium and potassium were kept in normal levels. CPB with perfusion cooling was performed for 30 to 40 minutes, and was maintained during 5 minutes after reaching 19°C, before initiating 90 minutes of deep hypothermic circulatory arrest (DHCA). The head and the ischemic leg were packed with ice during DHCA.

Following DHCA, CPB was restarted at a pressure of 20 mm Hg and slowly increased to 40 to 60 mm Hg. All pigs were then rewarmed to 37°C, by rewarming blood and the heating–cooling blanket with a 10°C gradient over rectal temperature. The heart was defibrillated at 30°C, and all animals were started on 5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of dopamine. Dopamine was then either increased or

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Table 2. Hemodynamic Variables

	No.	MAP mm Hg	Heart Rate	MPAP mm Hg	LAP mm Hg	Cardiac Output (L/min)	LVSWI	SVR	PVR	Dopamine Postop (min)
Baseline										
No DHCA	2	67	107	15	9	3.9	28	1200	143	
No treatment	5	66 ± 10	94 ± 12	13 ± 2	6 ± 2	4.2 ± 1.8	33.4 ± 17.4	1327 ± 492	153 ± 78	
HOE 642 pretreatment	5	61 ± 19	105 ± 12	8 ± 1	4 ± 1	4.2 ± 1.2	28 ± 10	1153 ± 244	89 ± 15	
After CPB										
No DHCA	2	53	150	18	7	3.2	12	1015	130	0
No treatment	5	68 ± 19	144 ± 28	20 ± 7	5 ± 4	4.2 ± 1.5	25 ± 10	1195 ± 499	343 ± 207	134 ± 61
HOE 642 pretreatment	5	74 ± 16	142 ± 18	21 ± 4	4 ± 1	4.7 ± 1.9	28 ± 10	1434 ± 778	352 ± 155	87 ± 54

CPB = cardiopulmonary bypass; DHCA = deep hypothermic circulatory arrest; HOE 642 = cariporide; LAP = left arterial pressure; LVSWI = left ventricular stroke work index; MAP = mean arterial pressure; MPAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; SVR = systemic vascular resistance.

weaned after discontinuation of CPB, to keep systolic arterial pressure above 70 mm Hg.

After the hemodynamic measurements, protamine was given and all cannulas were removed. Bleeding was controlled, the vessels were repaired, and all wounds were closed. Animals were then allowed to regain consciousness, extubated, and underwent neurologic assessment at 4, 6, and 24 hours. After extubation, buprenorphine (0.02 mg/kg intramuscularly) was administered for postoperative pain. Animals were euthanized with 30 mg/kg pentobarbital and potassium chloride after the last neurologic assessment.

Experimental Groups

CONTROLS. To distinguish the effects of extracorporeal circulation alone without ischemia, two pigs underwent cooling to 19°C and immediate rewarming to 37°C.

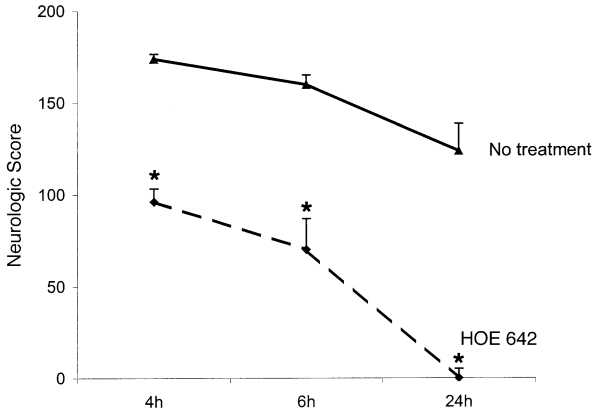

Fig 1. Neurologic evaluation of the deep hypothermic circulatory arrest groups that received no treatment (black line) *and Cariporide* (HOE, dashed line)*, at 4, 6, and 24 hours postcardiopulmonary bypass. Results are expressed as mean ± standard error of the mean* (error bars)*. Scoring system is shown in Table 1.* *p = *0.02.*

NO TREATMENT. Ten pigs underwent 90 minutes of deep hypothermic circulatory arrest at 19°C.

CARIPORIDE PRETREATMENT. In 5 pigs, 5 mg/kg cariporide was administered in the blood prime at the start of cooling, and each underwent 90 minutes of DHCA.

HEMODYNAMIC MEASUREMENTS. Hemodynamic measurements were made before and 30 minutes after discontinuing CPB. Cardiac output was determined by repetitive central venous injections of 4°C saline solution into the Swan-Ganz catheter.

BIOCHEMICAL ANALYSIS. Internal jugular blood samples were taken 5 minutes after initiating CPB (baseline), at 19°C before initiating DHCA, before resuming CPB, and at 4 and 24 hours post-CPB.

CONJUGATED DIENES. As a marker of oxidant-mediated lipid peroxidation, conjugated dienes (CD) levels were determined spectrophotometrically, after chloroform–methanol 2:1 (vol/vol) extraction as previously described [8], and expressed as absorbance at a wavelength of 240 nm/0.5 mL plasma.

CREATININE KINASE AND SERUM ASPARTATE AMINO TRANSFERASE. Cellular injury was determined by measuring creatinine kinase (CK) and serum aspartate amino transferase (AST) activity by the UV-spectrophotometric method (Sigma Chemical Co., St. Louis, MO) and expressed as U/mL plasma.

NITRIC OXIDE. Nitric oxide (μmoles/L) was determined as its spontaneous oxidation products, nitrite and nitrate, which were converted to nitric oxide and quantitated by a chemiluminescence assay using a nitrogen oxides analyzer (DASIBI Environmental Corp., Model 2108, Glendale, CA).

ENDOTHELIN-1. Endothelin-1 (ET-1) levels (pg/mL) were determined after sample purification (Ethyl C2 Amprep minicolumns, Amersham Pharmacia Biotech, Piscataway, NJ) by an Enzyme Immunometric Assay (ACE EIA kit, Cayman Chemical Company, Ann Arbor, MI) based on a double antibody "sandwich" technique.

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Table 3. Postoperative Neurologic Evaluation

	No treatment	HOE 642 pretreatment
Level of consciousness		
Normal	2 (40%)	5 (100%)
Cloudy	3 (60%)	0
Motor/sensory function		
Abnormal positioning	5 (100%)	0
Behavior		
Drinking	0	5 (100%)
Chewing	5 (100%)	5 (100%)
Sitting	2 (40%)	5 (100%)
Standing	(20%)	5 (100%)
Walking	1 (20%)	5 (100%)

HOE 642 = Cariporide.

NEUROLOGIC INJURY. Neurologic assessment was performed in all animals at 4 and 6 hours postanesthesia. Five pigs in each group were followed 24 hours. A commonly used neurologic scale developed at the University of Pittsburgh uniformly scored neurologic deficit (Table 1) [9]. In neurologic deficit scoring, five general components of the neurologic examination are evaluated and a score of 100 is assigned to each category. A total score of 500 indicates brain death, whereas a score of 0 is normal. Neurologic deficit scoring was agreed upon by two members of the laboratory team. When a different score was reported, the mean value was recorded for evaluation. All animals were closely evaluated for seizure activity.

STATISTICAL ANALYSIS. All data are expressed as mean ± standard deviation. Statistical analysis of data within and between groups was performed with multiple analysis of variance followed by application of the nonparametric Wilcoxon test. Changes within and between groups were considered statistically significant when the p value was less than 0.05.

Results

Comparability of Experimental Groups

There were no statistical differences between untreated and pretreated groups in cooling (41 ± 8 minutes and 41 ± 9 minutes, respectively) or rewarming time (82 ± 17 minutes and 91 ± 13 minutes, respectively). All animals remained in stable condition through out the experimental procedure. Hemodynamic variables were similar in both groups before and after CPB (Table 2). Dopamine perfusion was required in all animals (range 49 to 240 minutes post-CPB) with no differences among ischemic groups. Oxygen administration after extubation was required in most animals, with no differences between groups.

Postoperative Neurologic Outcome

Extubation time after weaning from anesthesia was similar in all groups. Animals that underwent CPB, cooling to 19°C, and immediate rewarming with no DHCA presented no neurologic deficits at 4 hours postanesthesia, although behavior such as sitting, standing, or walking was not present and each presented a cloudy level of consciousness. Neurologic recovery was almost normal

Table 4. Biochemical Data

	PreCPB	PreCA	PostCPB	4h postop	24h postop	*P* Between Groups	*P* Within Group (time)	Interaction Time Group
Conjugated Dienes (A/0.5 mL)						<0.01	<0.01	<0.01
No DHCA	0.89 ± 0.11	0.87 ± 0.19	1.00 ± 0.18	0.95 ± 0.14	0.97 ± 0.03			
No treatment	0.86 ± 0.07	0.84 ± 0.08	1.70 ± 0.10	1.58 ± 0.10	1.18 ± 0.05			
HOE 642 pretreatment	0.92 ± 0.04	0.85 ± 0.04	1.38 ± 0.06	1.26 ± 0.05	1.11 ± 0.21			
AST (U/mL)						<0.01	<0.01	<0.01
No DHCA	29 ± 1	33 ± 1	86 ± 22	107 ± 22	72 ± 4			
No treatment	29 ± 3	45 ± 4	265 ± 38	242 ± 33	179 ± 11			
HOE 642 pretreatment	33 ± 4	41 ± 5	195 ± 8	169 ± 9	126 ± 7			
CK (U/mL)						<0.01	<0.01	<0.01
No DHCA	385 ± 35	355 ± 35	638 ± 344	3715 ± 686	2032 ± 117			
No treatment	376 ± 37	381 ± 87	5153 ± 116	6257 ± 152	4317 ± 416			
HOE 642 pretreatment	401 ± 43	354 ± 28	3850 ± 193	4168 ± 109	2357 ± 322			
Endothelin-1 (pg/mL)						<0.01	<0.01	<0.01
No DHCA	0.95 ± 0.07	0.85 ± 0.07	0.90 ± 0.00	1.05 ± 0.07	0.90 ± 0.14			
No treatment	0.90 ± 0.07	0.81 ± 0.09	2.12 ± 0.19	1.88 ± 0.26	1.55 ± 0.13			
HOE 642 pretreatment	0.94 ± 0.05	0.84 ± 0.05	1.44 ± 0.11	1.32 ± 0.13	1.08 ± 0.08			

AST = aspartate amino transferase; CA = circulatory arrest; CK = creatine kinase; CPB = cardiopulmonary bypass; DHCA = deep hypothermic circulatory arrest; HOE 642 = Cariporide.

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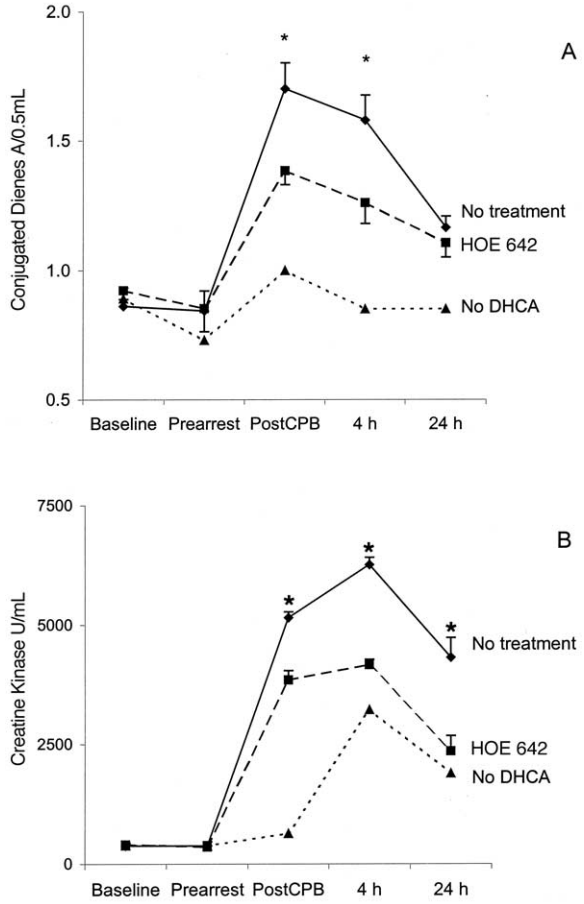

Fig 2. (A) Oxidant-mediated lipid peroxidation measured as conjugated dienes, and (B) creatinine kinase plasma levels in internal jugular vein at different stages of the experiment. *p < 0.05 *between groups. Values are mean ± standard error.* (CPB = *cardiopulmonary bypass;* HOE = *Cariporide;* DHCA = *deep hypothermic circulatory arrest.)*

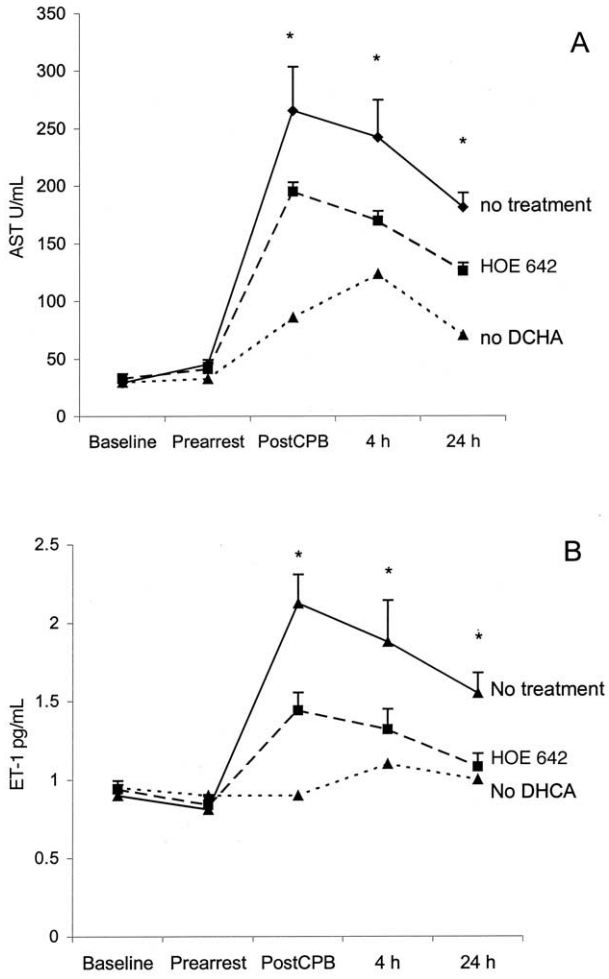

Fig 3. (A) Plasma levels of aspartate amino transferase (AST) *and (B) endothelin-1* (ET-1) *throughout the experiment.* *p < 0.05 *between ischemic groups. Values are mean ± standard error.* (CPB = *cardiopulmonary bypass;* HOE = *Cariporide;* DHCA = *deep hypothermic circulatory arrest.)*

at 6 hours, and related to only unbalanced walking, and was considered normal at 24 hours (Neurologic score is represented in Table 1).

Animals that underwent 90 minutes of DHCA presented with major neurologic deficits that were maintained at 24 hours. Abnormalities included inability to stand, walk, or drink, except for one pig that could stand.

In contrast, animals with 90 minutes of DHCA and cariporide pretreatment presented a similar recovery to animals with no DHCA: each of them could walk and feed themselves at 24 hours. Figure 1 and Table 3 show that the neurologic score between DHCA groups was statistically significant at 24 hours.

Biochemistry Analysis

None of the variables measured were modified by CPB and cooling to 19°C (Table 4). CD remained within base line levels in pigs not undergoing DHCA. In the untreated animals, a twofold rise in plasma levels was observed after rewarming. Conversely, pretreated animal levels were significantly lower than untreated animals post-CPB (Fig 2A). Indicators of cellular injury also rose after ischemia. Myocyte membrane disruption, measured as CK levels, rose in all groups, probably by limb ischemia owing to femoral cannulation (Fig 2B), and were higher after DHCA than in the control group. Differences between ischemic groups were also significant, with the lowest levels in the pretreated group. Similarly, post-CBP levels of AST were raised, with highest intergroup levels in the DHCA untreated animals (Fig 3A).

CPB and cooling to 19°C and rewarming did not raise vascular constrictor ET-1 from baseline levels. In contrast, DHCA raised ET-1 by twofold and it remained high at 24 hours (Fig 3B). In pretreated animals, ET-1 rose significantly less during the first 4 hours postoperative, and returned to baseline levels at 24 hours. Nitric oxide levels remained constant throughout the experiment in all groups.

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Comment

Deep hypothermic circulatory arrest is increasingly used as a surgical adjunct for an array of complex surgical procedures in children and adults. Concern about brain intolerance to anoxia has limited ischemic intervals to 45 to 60 minutes, despite cooling to 18° to 20°C [10]. This caution is undertaken because even shorter ischemic intervals cause neurologic damage [11, 12]. The pathophysiology of ischemic–reperfusion cerebral injury is uncertain, but excitatory amino acids may become neurotoxic, and calcium overload that is enhanced by NHE activity may produce neuron death by allowing activation of reactive oxygen species and disrupting the mitochondrial membrane [1].

Our study tested NHE inhibition before ischemia, and this treatment dramatically improved early neurologic recovery, suggesting that increased NHE activity contributes to brain damage after circulatory arrest. Animals pretreated with the NHE inhibitor cariporide exhibited a better whole-body protection from ischemia, shown by the return to control levels of several measured biochemical markers of cellular injury that included CK (myocyte injury), AST (global cell injury), and ET-1 (endothelium).

These salutary effects parallel previous reports of global [13] and selective NHE protection in heart, endothelium, platelets, liver, and brain [6, 14–17]. Of most importance was that the clinical assessment of these pigs showed total preservation of neurologic function after 90 minutes of DHCA, an ischemic time described to induce almost 100% of neurologic events [18]. The 24-hour time frame of postoperative evaluation, without histologic study, did not allow us to distinguish if the preliminary finding of complete recovery reflected very rapid improvement from brain stunning or avoided an irreversible brain infarction.

NHE activation during ischemia is mainly attributed to anaerobic-induced acidosis, although multiple signaling proteins have been described to stimulate the exchanger [19]. Six NHE subtypes have been described, with NHE-5 as the most common in neuronal tissue. We did not study prevailing mechanisms of action, but will summarize NHE interactions in ischemic tissue. As an *ATP independent* antiporter, NHE over-function during ischemia leads to sodium accumulation by expelling protons. The main pathways of intracellular sodium extrusion fail to limit sodium overload, as the *energy dependent* sodium–potassium pump is unable to work without ATP availability. Consequently, the high intracellular sodium concentration gradient activates the *ATP independent* sodium–calcium exchanger in a reverse mode to allow sodium extrusion, and thus, calcium accumulation [19]. Although unproven, we suspect that preischemic NHE inhibition protects the cell from calcium-induced injury by controlling cellular ion unbalance, resulting in full clinical neurologic recovery from ischemia–reperfusion. These actions matched this beneficial action in other measured cellular types [6, 14–17].

We used α-stat strategy to match clinical events in a circulatory arrest trial [20]. Studies by Jonas [21] show the benefits of pH-stat, with improved cerebral flow, slower brain desaturation during initiation of circulatory arrest, and luxury perfusion during rewarming. Although such reperfusion benefits of pH-stat may conceptually exert positive effects on brain calcium entry, our prior cardiac ischemia–reperfusion studies show that pH per se did not change myocardial damage [22]. Conversely, cariporide was effective at both α-stat and pH-stat blood pH status after cardiac ischemia–reperfusion studies [22], so that brain ischemia comparisons must now be made after longer DHCA intervals.

Our whole-animal studies enhance prior reports where cerebral NHE inhibition in brain ischemia–reperfusion is associated with better neuronal energy state preservation and lessened infarct-area edema [6, 23–25]. Of most importance was that gerbils received NHE inhibition during reperfusion and showed improved consciousness recovery after transient ischemia. The solid, reproducible, and complete neurologic recovery was a critical finding in our experiment; however, we recognize the limitations of a neurologic score, especially the inability to determine neurocognitive defects in pigs [26].

Our failure to extend our observations beyond 24 hours, do histologic analyses, or check for apoptosis, limits any long-term conclusions beyond those of neurologic normality. However, the central clinical time frame guideline is progressive improvement, so that we suspect that our treatment markedly limited brain damage [27].

Future studies will search for quantification of histology and apoptosis events, as these measurements will be introduced when longer ischemic intervals produce adverse clinical neurologic findings. Experimentally, NHE inhibition also diminishes cerebral endothelium postischemic dysfunction [15] and attenuates leukocyte adhesion during reperfusion [24]. Our finding of normal recovery of endothelin levels is in concert with these findings. Clinically, heart surgery patients who underwent extracorporeal circulation presented lower serum levels of S-100B and neurone-specific enolase when pretreated with cariporide, suggesting better preservation of the blood–brain barrier [28].

Our hypothesis is that anaerobic-induced proton accumulation triggers NHE stimulation and ultimately causes intracellular calcium overload. This is supported by previous studies showing that avoidance of an extracellular–intracellular pH gradient during ischemia by artificial acidification of the extracellular space will inhibit NHE and delay calcium entry [29, 30]. Also, hyperglycemic neurons delay calcium influx, probably by providing additional substrate for (anaerobic) ATP production and retarding proton accumulation [1]. These reports indicate that proton accumulation may be a main cause of calcium entrance. Pharmacologically blocking the NHE maintains intracellular anaerobic acidification by preventing proton extrusion and thereby limits calcium entrance. Although edema is limited by the inhibition of the main pathway of sodium into the ischemic cell, we did not measure

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brain water levels to document this presumed advantage.

We believe NHE inhibition may become an important factor that protects the brain against ischemic insult following reperfusion in a manner that parallels the benefits in the heart, as shown by our studies and those of other research groups [14, 31, 32]. This study differs from others, as our use as pretreatment differs from our prior studies that tested how neurologic injury in this same experimental model could be favorably changed by altering the reperfusate by filtering white cells, platelets, and complement during reperfusion [33].

The brain recovery in this study was complete and similar to that achieved by reperfusion management alone, but important systemic differences were observed. Aside from confirming the pretreatment benefits of NHE inhibition, the global effects of an intravenous preischemic intervention allowed better recovery of CK, AST, and endothelin than available when the treatment was restricted to focal white cell, platelet, and complement limitation during reperfusion into the carotid vessels [33].

Our studies did not differentiate between pretreatment and reperfusion management, because NHE inhibition was present during both intervals. We suspect that NHE inhibition will add to the benefits of isolated reperfusion management, but this must be tested, especially with longer ischemic time intervals. We suspect that neurologic damage is predominantly related to damage caused during reperfusion. Our suspicion is based upon the hypothesis that NHE inhibitor protection from the ischemic injury prevented neurons and endothelium from triggering reperfusion injury to limit reoxygenation damage and leukocyte adhesion and activation. We suspect that previous reports associating NHE inhibition to lower leukocyte-induced injury during reperfusion misinterpret the true cause-and-effect of NHE blockage [24].

Further data are needed before clinical applications of cerebral NHE inhibition can be launched, such as dose–response studies to maximize the therapeutic window while limiting possible adverse effects, and histopathologic studies must be done to search for late apoptosis. Nevertheless, confirmation of the benefits of NHE inhibition may open a therapeutic door to many situations in which cerebral ischemia is anticipated, such as carotid surgery, elective or emergent aortic aneurysm repair, cardiac surgery, and surgical procedures in adults and children that require deep hypothermic circulatory arrest. Furthermore, the reported benefits of cerebral protection when NHE inhibition is achieved during reperfusion expand its possible clinical usage to stroke and patients undergoing cardiopulmonary resuscitation [6, 24, 25].

We acknowledge that our study has limitations. We did not measure anticipated calcium, sodium, or water concentrations in brain tissue to confirm the anticipated ionic results of NHE inhibition. However, our ultimate goal was to record brain function. Histologic analyses are useful, but perhaps less meaningful than defining the outcome that is linked to how any test measures up against the desired neurologic outcome of complete recovery.

Our results may indicate only a faster neurologic recovery in an injury that may cause progressive cerebral edema so that future magnetic resonance imaging studies are needed for sequential comparison. Although many studies suggest the neurologic injury in the nontreated animals is permanent, graded improvement from brain stunning can occur so that longer follow-up is needed, especially when early evidence of damage is confirmed by clinical examination.

We conclude that NHE inhibition before ischemia limits neurologic ischemia–reperfusion injury, probably by better control of sodium, water, and calcium overload. We suspect NHE function plays an important role in global and more specifically in brain ischemia–reperfusion damage.

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DISCUSSION

DR JOHN MAYER (Boston, MA): I think this is really exciting work. I have a couple of questions.

First of all, in regard to the sodium–hydrogen exchanger mechanism, is that ubiquitously distributed throughout the CNS and throughout the body? I ask this because we know that certain areas of the CNS may be more vulnerable to ischemia–reperfusion than others. Is there any variation known in various parts of the CNS? And if this is applicable to CNS protection, is it also applicable to renal or cardiac or other kinds of protection?

The second question is, I noticed in your methods you have used an alpha-stat strategy versus a pH-stat strategy. The obvious question then is what impact do you think your pH strategy might have had on your results? Can you speculate on what the effects might be in a situation where you use pH-stat.

DR CASTELLÁ: Well, there are 6 isoforms described. Number 5 is the one more common in the central nervous system. Number 1 is the one most common in the heart. And I am not certain if there are very many differences between the neurologic tissues concerning distribution of the isoform number 5. Although we know that in the hippocampus studies, the most sensitive place of tissue within the central nervous system, the exchanger is active. So we can say that in the most sensitive tissue for ischemia, the exchanger is there.

About the pH-stat or the alpha-stat strategy, I can say from new experiments that we have performed after these studies, we know that the pH-stat strategy, by having lower pH during reperfusion, has much better results. And therefore, for example, using the same experimental model, we saw that the scoring of animals using pH-stat strategy was almost normal. So therefore, we think that either it can be additive, the results with cariporide and the pH-stat, or the pH-stat by itself, by lowering the pH, can be useful for this.

DR SCOTT M. BRADLEY (Charleston, SC): Does the administration of cariporide have any hemodynamic effects that you noted, or does it have any other negative side effects that could limit its use?

DR CASTELLÁ: Since it was given systemically, we could expect some hemodynamic results. And we saw that the levels of dopamine that we had to use were lower in the pretreated group. The necessity for dopamine was up to 87 minutes after cardiopulmonary bypass in the treated group and up to 137 minutes with no cariporide. And also, cardiac output was slightly higher in the pretreated group, it was 4.7 per liter minute mean against 4.2. But none of these results were significant.

And about side effects, we did not see any side effects in the treated group. We have to say though that we gave a low dose, 5 mg per kilo. We give it in one shot, only 5 minutes before

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cooling. So we did not give constant pre- or postoperative perfusion as it has been in different clinical trials.

DR ANDREW C. FIORE (St. Louis, MO): Did you make any effort to look at the actual brain temperature in these animals to be sure that it was the same in both groups?

DR CASTELLÁ: No. Since our main goal was to assess the clinical brain function, we could not measure calcium inside thecell or we could not measure temperature or we could not do anything that could harm during the postoperative period.

DR BRADLEY: I think that the slide you showed of the neurologic outcomes had two curves on it. What did the curve for the animals that had no circulatory arrest look like? Asked another way, did your bypass approach without any circulatory arrest have any detectable neurologic effects?

DR CASTELLÁ: Well, yes, just the cardiopulmonary bypass and hypothermia by itself showed that at the 4 and 6 hours the neurologic score was not normal. But we have to take into account that this is after extubation and still with a little bit of anesthesia, we can say. But the score was zero at 24 hours. So they were also considered normal. They were all walking, drinking and behaving normally.

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