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Response of Brain Oxygenation and Metabolism to Deep Hypothermic Circulatory Arrest in Newborn Piglets: Comparison of pH-Stat and Alpha-Stat Strategies

Scott D. Markowitz, MD, Alberto Mendoza-Paredes, MD, Huiping Liu, PhD, Peter Pastuszko, MD, Steven P. Schultz, MD, Gregory J. Schears, MD, William J. Greeley, MD, David F. Wilson, PhD, and Anna Pastuszko, PhD

Department of Anesthesiology and Critical Care Medicine, The Children's Hospital of Philadelphia; Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; Department of Surgery, University of Oklahoma, Oklahoma City, Oklahoma; Department of Anesthesiology, University of Miami, Miami, Florida; Department of Anesthesiology and Critical Care, Mayo Clinic, Rochester, Minnesota

Abstract

Background—To determine the effect of pH-stat as compared with alpha-stat management on brain oxygenation, level of striatal extracellular dopamine, phosphorylation, and levels of protein kinase B (Akt) and cyclic adenosine 3', 5'-monophosphate response element-binding protein (CREB), and levels of extracellular signal-regulated kinase (ERK)1/2, Bcl-2, and Bax in a piglet model of deep hypothermic circulatory arrest (DHCA).

Methods—The piglets were placed on cardiopulmonary bypass (CPB), cooled with pH-stat or alpha-stat to 18°C, subjected to 90 minutes of DHCA, rewarmed, weaned from CPB, and maintained for two hours recovery. The cortical oxygen was measured by: quenching of phosphorescence; dopamine by microdialysis; phosphorylation of CREB (p-CREB), ERK (p-ERK) 1/2, Akt (p-Akt), and level of Bcl-2, Bax by Western blots.

Results—Oxygen pressure histograms for the microvasculature of the cortex show substantially higher oxygen levels during cooling and during the oxygen depletion period after cardiac arrest (up to 15 minutes) when using pH-stat compared with alpha-stat management. Significant increases in dopamine occurred at 45 minutes and 60 minutes of DHCA in the alpha-stat and pH-stat groups, respectively. The p-CREB and p-Akt in the pH-stat group were significantly higher than in the alpha-stat group ($140 \pm 9\%$, $p < 0.05$ and $125 \pm 6\%$, $p < 0.05$, respectively). There was no significant difference in p-ERK1/2 and Bax. The Bcl-2 increased in the pH-stat group to $121 \pm 4\%$ ($p < 0.05$) compared with the alpha-stat group. The ratio Bcl-2:Bax increased in the pH-stat group compared with the alpha-stat group.

Conclusions—The increase in p-CREB, p-Akt, Bcl-2, Bcl-2/Bax, and delay in increase of dopamine indicated that pH-stat, in the piglet model, prolongs "safe" time of DHCA and provides some brain protection against ischemic injury.

Prolonged deep hypothermic circulatory arrest (DHCA) contributes significantly to neurological and neuropsychological deficits and remains a major cause of postoperative morbidity and mortality. Hence, there is a pressing need to provide strategies for reducing brain

injury and developmental abnormalities caused by DHCA. Over the last few years, pH-stat versus alpha-stat blood gas management during cardiopulmonary bypass (CPB) has been investigated in attempts to establish which of these techniques better protects the brain from DHCA-dependent injury. The published data, however, do not provide strong evidence in favor of either of these methods.

Alpha-stat has been reported to give better preservation of intracellular pH and enzyme activity as well as maintenance of cerebrovascular autoregulation and cerebral flow-metabolism coupling than pH-stat. It has also been reported that alpha-stat blood gas management is more neuroprotective, presumably due to fewer cerebral microemboli [1–3]. Neurologic outcomes have been suggested to be better with the alpha-stat strategy in adult patients undergoing CPB, whereas in infants pH-stat strategy may be more beneficial [4]. On the other hand, pH-stat management has been reported to provide greater cerebral blood flow, more homogeneous cooling, greater reduction in cerebral oxygen consumption, and higher tissue oxygen pressures during cooling. The use of pH-stat blood gas management during cooling has been shown to decrease brain oxygen consumption by 30% to 40% below the levels achieved with alpha-stat management [5,6]. In 1998, Kurth and colleagues [7] showed that pH-stat increased the rate of homogenous brain cooling during CPB due to increased cerebral flow. Then, Priestley and colleagues [8] reported that pH-stat improved neurologic outcome in piglets undergoing 90 minutes of DHCA in a survival model. Hiramatsu and colleagues [9] had already reported that, in neonatal piglets, pH-stat resulted in better preservation of cellular oxygen delivery and improved recovery of cerebral high-energy phosphates as well as intracellular pH during reperfusion [9]. However, pH-stat has also been reported to result in loss of cerebrovascular autoregulation and to increase the risk of cerebral microemboli [5–7].

The purpose of this study was to compare differences in brain oxygenation and metabolism between pH-stat and alpha-stat management in a piglet model of DHCA. Our hypothesis was that pH-stat management would result in increased brain oxygenation, delay in the increase of extracellular striatal dopamine (DA), and a pattern of alterations in the activities of selected regulatory proteins consistent with better biochemical outcome. Striatal extracellular dopamine was measured as a sensitive marker for adequate brain oxygenation and a mediator of neuronal injury, particularly within the striatum. The regulatory proteins, cyclic adenosine monophosphate (AMP) response element-binding protein (CREB), protein kinase K (Akt), extracellular signal-regulated kinase (ERK1/2), Bcl-2, and Bax, were selected for measurement based on their participation in pathways contributing to the death or survival of neurons after hypoxic-ischemic insult.

Materials and Methods

Animal Model

A total of 14 newborn piglets, two to four days of age (1.4 to 2.5 kg) were randomly assigned to either alpha-stat (n = 7) or pH-stat (n = 7) groups. The alpha-stat group was the control group. After induction with halothane, a tracheotomy was performed to maintain stable and reliable control of the airway. The piglets were then mechanically ventilated and anesthesia was maintained with isoflurane and fentanyl while pancuronium was used to facilitate mechanical ventilation. Femoral venous and arterial cannulae were placed for the collection of samples and arterial pressure monitoring. The animal's head was placed in a stereotaxic holder, the scalp removed, and an 8-mm-diameter window was made over the right parietal hemisphere for measuring cerebral cortical oxygen pressure. Another window was created over the left parietal hemisphere and a microdialysis probe was stereotactically implanted into the left striatum. The correct placement of the probe was later confirmed during preparation of the specimen. The CPB protocol was started after a one-hour stabilization period and was followed by a two-hour recovery period, also under anesthesia. The animals were then euthanized with

4M KCl and the brain immediately dissected and frozen for later analysis. All animal procedures were in strict accordance with the National Institutes of Health, *Guide for the Care and Use of Laboratory Animals*, and have been approved by the local Animal Care and Use Committee.

DHCA Technique and Experimental Protocol

The protocols and techniques used during these studies duplicated those practiced in the clinical setting. Experimental animals were placed in the supine position and sternotomy performed for access to the heart and great vessels. The piglets were cannulated in the right atrium and ascending aorta. The CPB (at full flow of 150 mL/kg/ min) was instituted by four minutes of cooling and piglets were cooled over 30 minutes to a nasopharyngeal temperature of 18°C. Rectal temperature was also monitored to ensure even cooling and good arterial cannula placement. Supplementary topical ice packs were employed to ensure thorough and even cooling to the target temperature. Anesthesia was maintained during CPB using 1% isoflurane and fentanyl (additional 50 mcg/kg). Pancuronium 0.2 mg/kg was also used in the CPB prime. The hematocrit was maintained at 30% during CPB by pump prime makeup with porcine whole blood, albumin, and crystalloid.

Cooling was controlled to produce the appropriate arterial blood gas pH of 7.4 and partial pressure of carbon dioxide of 40 mm Hg in either the temperature corrected (pH-stat) or 37°C (alpha-stat) groups. Fresh gas flow (sweep) was typically 300 mL/minute for alpha-stat, and 1.5 L/minute with CO₂ 50 mL/minute for pH-stat. By 30 minutes, all piglets had achieved 18°C. At 30 minutes of cooling CPB was discontinued for 90 minutes of DHCA and venous drainage remained open to the CPB reservoir. After the DHCA period, CPB was restarted and the piglets rewarmed to 37°C using alpha-stat management for 30 minutes. The piglets were then weaned from CPB and maintained under anesthesia for a two-hour recovery period.

In the clinics, the period of circulatory arrest is limited to 45 to 60 minutes to minimize injury to the patient. In experimental animal models it is necessary to have sufficient injury for accurate and reproducible measurements, as this is essential to testing the efficacy of putative protective protocols. The assumption is made that the injury resulting from the longer arrest period (90 minutes) is representative of that occurring in the clinics, but progressed to where it can be accurately measured in every animal.

Measurement of Oxygen Pressure and Oxygen Distribution by the Oxygen-Dependent Quenching of Phosphorescence

Cerebral cortical oxygen pressure was measured using oxygen-dependent quenching of phosphorescence as described earlier [10,11]. Oxygen is measured in the microcirculation of approximately 300 mm³ of cortical tissue by the quenching of phosphorescence of an oxygen sensor, Oxyphor G2, dissolved in the blood plasma. The oxygen distribution as a function of blood volume, oxygen histogram, is calculated from the distribution of phosphorescence lifetimes in the sampled volume [12]. The value for each oxygen pressure is the mean $\pm$ standard error (SE).

Measurement of Extracellular Dopamine by In Vivo Microdialysis

The level of extracellular dopamine in the striatum was measured by in vivo microdialysis as described in earlier publications [13,14].

Western Blotting

The Western blots of CREB, ERK1/2, Bcl-2, Bax, and Akt were performed as described earlier [15,16]. The striatal membranes were incubated with specific antibodies for above proteins and beta-actin antibody served as a loading control.

Data Analysis

Autoradiographic films were analyzed using Scion Image software (National Institutes of Health Image). Each blot contained two sets of samples, one for an alpha-stat (control group) and another for the experimental group (pH-stat group). The data were normalized to the values obtained for the alpha-stat (control group) (assigned a value of 100).

All physiologic parameters, cortical oxygen pressure values, and levels of extracellular dopamine are expressed as means for seven experiments $\pm$ SE or SD. Statistical analysis was by the Student *t* test. A *p* value less than 0.05 was considered statistically significant.

Results

Effect of DHCA With Alpha-Stat Versus pH-Stat Management on Physiologic Parameters and Cortical Oxygen Pressure in Newborn Piglets

During cooling with alpha-stat management, the values of arterial pH, partial pressure of carbon dioxide, arterial (Paco₂), and partial pressure of oxygen, arterial (Pao₂) were 7.8 ± 0.02 , 28.6 ± 5.7 mm Hg, and 200 ± 48.6 mm Hg, respectively. When cooling was with pH-stat management, the arterial pH was 7.38 ± 0.03 ($p < 0.05$), a value significantly lower than with alpha-stat management. The Paco₂ was higher (39 ± 2.9 vs 28.6 ± 5.7 mm Hg; $p < 0.05$) in pH-stat than alpha-stat management, whereas the Pao₂ (236 ± 17.9 mm Hg) was not significantly different between the two.

The oxygen distributions in the cortex, measured before cooling (controls), were not significantly different for the two groups of piglets (Fig 1A). At the end of the 30-minute cooling period, cortical oxygenation was significantly higher with pH stat than with alpha stat management (Fig 1B). The higher oxygenation with pH stat management was also observed at 5 and 15 minutes after circulatory arrest (Figs 1C, 1D). The histograms are presented as the means $\pm$ SE, and significant differences are indicated where there is no overlap of the error bars. The fraction of the tissue volume with oxygen pressures less than 10 mm Hg was calculated from the mean of the histograms (Table 1). At the end of the 30-minute cooling period and after 5, 15, and 30 minutes of arrest were 6.8, 17, 36, and 38%, respectively, for the alpha-stat group whereas for the pH-stat group they were 0.3, 8.4, 27, and 33%, respectively.

Changes in Extracellular Striatal Dopamine During DHCA With Alpha-Stat or pH-Stat Management

The effects of DHCA with alpha-stat and pH-stat on the levels of extracellular dopamine are shown in Figure 2. Significant increases in extracellular dopamine occurred at 45 minutes of DHCA in the alpha-stat group but not until 60 minutes of DHCA in the pH-stat group.

Changes in the Levels and Phosphorylation of Selected Proteins Playing Major Roles in Hypoxic-Ischemic Injury or Cell Survival

The phospho-CREB immunoreactivity and phospho-Akt immunoreactivity in the striata of the animals after DHCA with pH-stat plus two hours of post-bypass recovery were found to be significantly higher than in the alpha-stat group ($140 \pm 9\%$, $p < 0.05$ and $125 \pm 6\%$, $p < 0.05$, respectively) (Fig 3). There was no significant difference in phospho-ERK1/2 immunoreactivity in the striata after DHCA in the two groups (Fig 3).

The Bcl-2 immunoreactivity was increased after DHCA with pH-stat to $121 \pm 4\%$ ($p < 0.05$) compared with the alpha-stat group and there was no significant change in the N-Bax immunoreactivity between groups (Fig 4). Therefore, the Bcl-2:Bax ratio was higher in the pH-stat group as compared with the alpha-stat group.

Comment

The purpose of the present study was to determine the responses of the cortical oxygen pressure and brain metabolism to DHCA after cooling using pH-stat as compared with alpha-stat management. The absence of clinical data showing an outcome advantage with a particular pH strategy has not prevented practitioners from choosing sides in the controversy. Our unique ability to measure tissue-available oxygen in the cerebral microcirculation and to correlate this to an accepted marker of striatal hypoxic injury (extracellular dopamine) adds meaningful data to the discussion of this important technical question.

The results shown, in agreement with our early study [15], that the oxygen pressure decreased significantly during cooling with alpha-stat management and after 30-minute DHCA there was a significant increase in extracellular dopamine. It was also described that after 90 minutes of DHCA with alpha-stat management and two hours of recovery, the N-Bax levels increased to $295 \pm 15\%$ of control, Bcl-2 levels decreased to 32% of control, and phosphorylation levels of Akt and CREB did not change [16,17].

During cooling and first five minutes of DHCA, cortical oxygenation with pH-stat was significantly higher than with alpha-stat management as shown by the shift in the histograms to higher oxygen pressures and the decreased volumes of tissue with oxygen pressures less than 10 mm Hg. This improvement in brain oxygenation during the cooling with pH-stat management is consistent with the higher level of CO₂ causing vascular dilation, accompanied by increase in cerebral blood flow and blood content. This contrasts with alpha-stat management where the lower CO₂ and more alkaline pH is expected to lower cerebral blood flow and to increased hemoglobin oxygen affinity. Thus, pH-stat management results in a greater content of oxygenated hemoglobin in the brain at the start of DHCA, prolonging the time required to completely deplete the oxygen supply and thereby the time before cellular energy metabolism fails due to lack of oxygen. This is consistent with reports that when pH-stat was used, as compared with alpha-stat, cerebral ATP and phosphocreatine returned to normal more rapidly and the redox state of cytochrome aa3 was improved [9].

To determine if the observed differences in brain oxygenation and pH affect longer term regulation of brain metabolism, we measured the levels of extracellular striatal dopamine and the activity of selected regulatory proteins having important roles in neuronal injury or survival after ischemic-hypoxic conditions. The statistically significant increase in extracellular dopamine in the striatum during DHCA with pH-stat management was delayed about 15 minutes as compared with the alpha-stat strategy. The extracellular dopamine level within the striatum provides a very sensitive measure of the adequacy of brain oxygenation [18]. A limitation of these studies was that the oxygen pressures were measured in the cortex, whereas the extracellular level of dopamine was measured in striatum. However, if the massive release of dopamine is correlated with the failure of cellular energy metabolism, then failure of striatal energy metabolism occurs after a significantly longer period of circulatory arrest for pH-stat than for alpha-stat management. This is consistent with the work of Aoki and colleges [19] showing that regional blood flow in the basal ganglia, midbrain, and cerebellum was significantly higher in the pH-stat group during cooling.

The delay in dopamine release during DHCA with pH-stat as compared with alpha-stat may be an indicator of a delay in neuronal injury. It is generally accepted that the increase in

extracellular dopamine can play a detrimental role in the development of ischemic cell damage in the striatum (see review [18]).

Our data show that during DHCA with alpha-stat management, occurrence of a massive increase in extra-cellular striatal dopamine correlates with the end of the “safe” period for cardiac surgery. It can therefore be concluded from the presented dopamine measurements that using pH-stat management can increase by about 15 minutes the “safe” period of DHCA.

To further confirm that pH-stat management has a beneficial effect on brain metabolism as compared to alpha-stat management, the levels and phosphorylation of selected proteins were determined. The CREB, Akt, ERK1/2, Bcl-2, and Bax were chosen for measurement based on evidence that they are important regulators of cell survival and death after cerebral ischemic injury. The data show that using pH-stat as compared with alpha-stat resulted in a small but significant increase in the phosphorylation and level proteins which play important roles in cell survival (Akt, CREB, and Bcl-2), without altering the level of Bax (one of the major apoptotic proteins).

Akt, a potent kinase, targets several key proteins to keep cells alive, including apoptosis regulators and transcription factors [20,21]. Akt has also been shown to cause the phosphorylation of CREB. Several studies involving overexpression of dominant-negative CREB suggest a role for CREB as a survival factor in various cellular models [22–24]. It has been shown that overexpression of CREB decreases apoptosis through upregulation of Bcl-2 expression [25,26].

Increase in phosphorylation of CREB is dependent on the severity of insult. Our earlier studies showed that there is an increase in the phosphorylation of CREB after low flow cardiopulmonary bypass and selective cerebral perfusion, procedures that provide continued low rates of perfusion of the brain, when compared with DHCA alone [15,16,27].

Similar to Akt and CREB, accumulating evidence indicates that overexpression of Bcl-2 provides protection against apoptosis [28] and ischemic neuronal death [29,30]. The Bcl-2 family is divided into two groups: anti-apoptotic members include Bcl-2 and proapoptotic members include Bax. The active form of Bcl-2 heterodimerizes with Bax and their ratio determines the cellular susceptibility to apoptotic stimuli. An increased ratio of Bax to Bcl-2 protein was shown in hypoxic and hypocapnic piglets, demonstrating an increased susceptibility to apoptosis in the hypoxic and hypocapnic newborn brains [31]. Our data show that while Bax did not change significantly, there was a significant increase in Bcl-2 levels in the pH-stat group as compared with the alpha-stat group; therefore, the ratio of Bcl-2/Bax was higher in pH-stat group. This is consistent with better neuronal survival when using this strategy as compared with the alpha-stat strategy.

It can be concluded that, in the newborn piglet model, using pH-stat management as compared with alpha-stat management should result in better outcome by providing significantly greater neuronal protection against ischemic injury and an increased “safe” period of DHCA.

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Fig 1. Effect of alpha-stat and pH-stat strategy on cortical oxygen pressure in newborn piglets during cooling and deep hypothermic circulatory arrest (DHCA). The histograms are for control (A), after 30 minutes cooling (B), 5 minutes after the start of DHCA (C), and 15 minutes after the start of DHCA (D). The oxygen pressures are corrected for the temperature of the blood at the time of measurement. The presented histograms are the means $\pm$ standard error for seven experiments. (■ = % [alpha-stat]; ○ = % [pH-stat].)

Fig 2.

The effect of deep hypothermic circulatory arrest (DHCA) with alpha-stat and pH-stat management on the extracellular concentrations of dopamine in striatum of newborn piglets. The three measurements of the dopamine during the pre-bypass period were averaged and the value considered as the baseline (100%). The results are means for seven alpha-stat and seven pH-stat experiments $\pm$ SD. ($p < 0.05$ for significant difference from alpha-stat values as determined by the Student t test; $\circ$ = pH-stat; $\blacksquare$ = alpha-stat.)

Fig 3.

Effect of deep hypothermic circulatory arrest (DHCA) with alpha-stat and pH-stat management on phosphorylated Akt, CREB, and ERK1/2 in the striatum of newborn piglets. (Top panel) Representative Western Blots for protein samples from DHCA with alpha-stat and pH-stat groups probed with antibodies against phosphorylated Akt (pAkt), phosphorylated CREB (pCREB), and phosphorylated ERK1/2 (pERK1/2). (Bottom panel) The means for DHCA with pH-stat ($n = 7$) $\pm$ SD, expressed in % to the corresponding values obtained for the alpha-stat group ($n = 7$). ($p < 0.05$ for significant difference from alpha-stat values as determined by the Student t test.)

Fig 4.

Effect of DHCA with alpha-stat and pH-stat management on N-Bax and Bcl-2 in the striatum of newborn piglets. (Top panel) Representative Western Blots for protein samples from alpha-stat and pH-stat groups probed with N-Bax or Bcl-2 antibodies. (Bottom panel) The means for DHCA with pH-stat (n=7) ± SD, expressed in % of the value obtained for alpha-stat (n=7). a $p < 0.01$ for significant difference from alpha-stat values as determined by Student t test.

Table 1

Effect of DHCA With Alpha-Stat and pH-Stat on Percent of the Measured Histogram With Oxygen Pressures Less Than 10 mm Hg

Experimental Conditions	Alpha-Stat	pH-Stat
Control	1.1	1.5
Cooling 15 min	2.9	0.6
Cooling 30 min	6.8	0.3
DHCA 5 min	17	8.4
DHCA 15 min	36	27
DHCA 30 min	38	33

These values are the sum of the mean values (Y values) for less than 10 mm Hg for each experimental condition $\times 100$.
DHCA = deep hypothermic circulatory arrest.