SURGERY FOR CONGENITAL HEART DISEASE

THE FREE RADICAL SPIN TRAP α -PHENYL-*TERT*-BUTYL NITRONE ATTENUATES THE CEREBRAL RESPONSE TO DEEP HYPOTHERMIC ISCHEMIA

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Objective: The aim of this study was to assess the role of reactive oxygen species in the impairment of cerebral recovery that follows deep hypothermic circulatory arrest. **Methods:** Twelve 1-week-old piglets were randomized to placebo (control group; $n = 6$) or 100 mg $\cdot$ kg⁻¹ intravenous α -phenyl-*tert*-butyl nitron, a free radical spin trap (PBN group; $n = 6$). All piglets underwent cardiopulmonary bypass, cooling to 18°C, 60 minutes of circulatory arrest followed by 60 minutes of reperfusion, and rewarming. Cerebral blood flow and metabolism were determined at baseline before deep hypothermic circulatory arrest and after 60 minutes of reperfusion. **Results:** In control animals, mean global cerebral blood flow (± 1 standard error) before circulatory arrest was 48.4 ± 3.6 mL $\cdot$ 100 g⁻¹ $\cdot$ min⁻¹ and fell to 25.1 ± 3.6 mL $\cdot$ 100 g⁻¹ $\cdot$ min⁻¹ after circulatory arrest ($P = .001$). Global cerebral metabolism fell from 3.5 ± 0.2 mL $\cdot$ 100 g⁻¹ $\cdot$ min⁻¹ before arrest to 2.2 ± 0.2 mL $\cdot$ 100 g⁻¹ $\cdot$ min⁻¹ after circulatory arrest ($P = .0002$). In the PBN group after circulatory arrest, the mean global cerebral blood flow and metabolism of 37.2 ± 4.9 and 3.6 ± 0.5 mL $\cdot$ 100 g⁻¹ $\cdot$ min⁻¹, respectively, were significantly higher than in the control group ($P < .05$). Recovery of cerebral blood flow in the PBN group was 78% of pre-arrest level compared with 52% in the control group ($P = .002$). Global cerebral metabolism after circulatory arrest was 100% of the pre-arrest value compared with 61% in the control group ($P = .01$). Regional recovery of cerebral metabolism in the cerebellum, brain stem, and basal ganglia was 131%, 130%, and 115%, respectively, of pre-arrest values in the PBN group compared with 85%, 78%, and 70% in the control group ($P < .04$). **Conclusions:** Reactive oxygen species contribute to the impairment of cerebral recovery that follows deep hypothermic circulatory arrest. The use of α -phenyl-*tert*-butyl nitron before the arrest period attenuates the normal response to ischemia and improves recovery by affording protection from free radical-mediated damage. (J Thorac Cardiovasc Surg 2000;119:305-13)

Free radicals are chemical species with one or more unpaired electrons in their outer orbital. This makes them unstable and highly reactive with nearby mole-

cules. As a result, free radicals are potentially dangerous to living tissue. They are formed during normal cerebral metabolism but cause injury only when they

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exceed the antioxidant defenses of the tissue. The extreme reactivity of free radicals means that they never accumulate to concentrations sufficient to allow direct observation. Addition of a free radical to a nitron, however, yields a nitroxide species, which can be detected by electron spin resonance spectroscopy.

The nitrones were developed in the 1960s specifically for the indirect detection of short-lived free radical species. One such nitron is α -phenyl-*tert*-butyl nitron, or PBN. Chemicals that can combine with free radicals to allow detection by electron spin resonance are known as "spin traps," and the combination of the free radical with the nitron is termed a "spin adduct." PBN was first described in the context of free radical spin trapping in 1968.¹ Although nitron spin traps have been used to detect free radicals since the 1960s, it was not until 20 years after this that consideration was given to their therapeutic potential. Neuroprotective properties of the nitrones were not described until the early 1990s.^{2,3}

At least three chemical species, superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl (OH) free radicals, are involved in oxygen-derived free radical damage in biologic systems. Oxygen-derived free radicals play a key role in the pathologic processes induced by cerebral ischemia, and the OH radical is the most important of the oxygen-derived free radicals in this context. Ischemia-reperfusion injury induces OH radical formation in brain microvessels.⁴ Subsequent free radical damage to the cerebral vasculature causes an increase in permeability of the blood-brain barrier,^{5,6} altered membrane ion transport activity,⁷ and enhanced neutrophil adhesion to the vascular endothelium.⁸

In addition to cerebral vascular injury, oxygen-derived free radicals induce membrane lipid peroxidation.⁹ Neuronal membranes are particularly susceptible to free radical attack because they are rich in polyunsaturated fatty acids. Potential consequences of damage to membrane lipid include changes in fluidity and permeability and in the orientation of proteins embedded in the bilayer of the plasma membrane and in other cellular endomembranes.¹⁰ Other important targets for free radical attack are proteins, amino acids, nucleic acid bases, carbohydrates, neurotransmitters, and DNA.¹¹ The resulting alterations in morphology and function are potentially lethal, causing neuronal death. The enhanced propensity of cerebral tissue to peroxidize, the fact that it is intricately and highly organized and carries out central information processing, plus the fact that neurons are postmitotic cells combine to make free radical damage a particularly serious problem in the brain.

The generation of free radicals after cerebral ischemia would appear to be central to the subsequent vascular and neuronal damage. Scavenging of these free radicals could offer enormous therapeutic benefit. The aim of this study was to use PBN to indirectly assess the role of free radicals in the impairment of cerebral recovery that is known to follow a period of deep hypothermic circulatory arrest (DHCA) in children.¹² The null hypothesis is that free radicals are not involved in the post-DHCA impairment in cerebral blood flow (CBF) and cerebral metabolism ($CMRO_2$) and that PBN given before cardiopulmonary bypass (CPB) will have no effect on cerebral recovery after 60 minutes of DHCA in the neonatal piglet.

Material and methods

Animal preparation. All animal experiments were conducted with the approval of the Animal Care and Use Committee of Duke University. The animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health (NIH publication 85-23, revised 1995) and were housed in the institution's NIH-approved animal facility before the experiments.

Twelve neonatal piglets (1-2 weeks old) were anesthetized with an intramuscular injection of ketamine ($50 \text{ mg} \cdot \text{kg}^{-1}$) and acepromazine ($15 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$). Intravenous methylprednisolone ($30 \text{ mg} \cdot \text{kg}^{-1}$) was administered via a 24-gauge cannula in the marginal vein of the pinna. Orotracheal intubation was performed and mechanical ventilation (Infant Ventilator, Sechrist Industries, Anaheim, Calif) was commenced to achieve arterial oxygen tensions of 150 to 250 mm Hg and carbon dioxide tensions of 35 to 45 mm Hg. The animals were paralyzed with intravenous pancuronium ($300 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$) and anesthetized with fentanyl ($100 \text{ } \mu\text{g} \cdot \text{kg}^{-1}$). Thereafter, anesthesia was maintained with a continuous infusion of fentanyl ($25 \text{ } \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). An 18-gauge cannula was placed in the descending aorta via the femoral artery for blood pressure monitoring and arterial blood sampling. The animal's temperature was monitored throughout the study by an indwelling nasopharyngeal temperature probe (Yellow Springs Instrument Co Inc, Yellow Springs, Ohio). Temperature was maintained at 36°C except for the period of induced hypothermia.

The heart was exposed through a median sternotomy. Cardiac instrumentation consisted of a 3F micromanometer (Millar Instruments, Inc, Houston, Tex) inserted into the superior vena cava for central venous pressure monitoring and placement of an 8-mm flow probe (Transonic Systems, Inc, Ithaca, NY) around the proximal pulmonary artery for cardiac output monitoring.

Sagittal sinus access. The animals were anticoagulated with intravenous heparin (500 IU/kg) before access of the sagittal sinus. A 1-cm strip of scalp was raised in the midline over the vertex of the skull. Two separate 2-mm burr holes

were made over the superior sagittal sinus for repeated sampling of sagittal sinus venous blood and continuous monitoring of sagittal sinus venous pressure with a 3F micro-manometer (Millar Instruments).

CPB and circulatory arrest. An 8F arterial cannula and a 20F venous cannula (DLP Inc, Grand Rapids, Mich) were inserted through purse-string sutures into the ascending aorta and the right atrium, respectively. CPB was commenced at a flow rate of $120 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. The pump-oxygenator system consisted of a Sarns nonpulsatile roller pump (Sarns Inc/3M Health Care, Ann Arbor, Mich) and a Medtronic Minimax PLUS hollow-fiber membrane oxygenator (Medtronic Inc, Anaheim, Calif). No arterial filter was used. The circuit was primed with heparinized fresh blood from a donor pig. Ringer's lactate and sodium bicarbonate solutions were added to the prime to achieve a hematocrit value of 0.25 and a pH of 7.4 at 37°C . The total prime volume was approximately 450 mL. The temperature of the perfusate was controlled with the integral heat exchanger in the venous reservoir of the oxygenator and a BIO-CAL 370 water bath system (Medtronic Bio-Medicus, Eden Prairie, Minn). Animals were cooled to a temperature of 18°C over a standard duration of 20 minutes by the circulation of ice water through the heat exchanger. At the end of the cooling period, the circulation was arrested and blood was drained from the animal. DHCA was therefore established and the aortic and right atrial cannulas were clamped. After 60 minutes of DHCA, the aortic and venous cannulas were unclamped. Perfusion was re-established at $120 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ with the perfusate initially at room temperature (20°C - 22°C). Rewarming was accomplished by circulating warm water through the heat exchanger in the venous reservoir. A nasopharyngeal temperature of 36°C was generally reached by 45 minutes of reperfusion. During cooling and rewarming, blood gases were managed according to the alpha-stat strategy. The arterial pH was maintained at 7.35 to 7.45 and carbon dioxide tension at 35 to 45 mm Hg, measured at 37°C and uncorrected for the temperature of the animal. Arterial oxygen tension was kept between 150 and 250 mm Hg and hematocrit between 0.23 and 0.28. Sodium bicarbonate (8.4%) was given when necessary but not immediately before CBF measurements. At the end of the study, the animals were killed by a bolus injection of fentanyl and cessation of CPB.

Measurement of CBF. CBF measurements were determined by the reference-sample, radiolabeled microsphere technique¹³ during CPB at 36°C . The technique described in the current study is the same as that used in previous reports from our laboratory.¹⁴ Suspensions of microspheres with a diameter of $15.5 \pm 0.1 \mu\text{m}$ (E.I. DuPont de Nemours & Company, Wilmington, Del) were made up in 10% dextran and 0.01% TWEEN 80 with 10^6 microspheres per milliliter. Two different isotopes were used in each piglet and these were chosen at random from the three different isotopes available (^{46}Sc , ^{103}Ru , and ^{95}Nb). For each flow measurement, 10^6 microspheres were injected into a side port of the arterial tubing 30 cm proximal to the aortic cannula over 30 seconds and washed through with 5 mL of warm saline solu-

tion. A reference blood sample was withdrawn from the distal aorta at a constant rate of $3 \text{ mL} \cdot \text{min}^{-1}$ with a Harvard syringe pump (Harvard Apparatus, South Natick, Mass) commencing 10 seconds before the microsphere injection and continuing for 2 minutes. At the end of the experiment, the brain was removed and divided into left and right cerebral hemispheres, basal ganglia, cerebellum, and brain stem (mid-brain, pons, and medulla oblongata). In addition, the kidneys were also removed for determination of renal blood flow. After measurement of fresh weights, the brain parts and kidneys were dissolved in 2 mol/L potassium hydroxide solution and analyzed, together with the reference blood sample, in a gamma counter (Auto-Gamma 5530; Packard Instrument Co, Meriden, Conn) to estimate the quantity of each type of radio-labeled microsphere present in the specimen. The withdrawal rate of the reference blood sample and the ratio of counts from a brain part to the reference blood sample allowed calculation of regional CBF. CBF measurements are expressed in milliliters per 100 g of brain per minute by normalizing for fresh tissue weight. The weighted sum of regional CBF allowed calculation of global CBF.

Cerebral perfusion pressure (CPP) was taken as the difference between the mean arterial pressure and the sagittal sinus venous pressure. Cerebral vascular resistance (CVR) was the ratio of CPP to global CBF (in milligrams of mercury per 100 g per minute per milliliter). Systemic vascular resistance was taken as the ratio between the systemic perfusion pressure and the total bypass pump flow rate (systemic vascular resistance = [mean arterial pressure – right atrial pressure] / [pump flow rate] in millimeters of mercury per 100 g per minute per milliliter).

Measurement of cerebral oxygen handling. Arterial and sagittal sinus blood samples were taken just before each microsphere injection for estimation of oxygen tension, carbon dioxide tension, oxygen saturation, pH, and base excess by means of a GEM-Stat blood gas/electrolyte monitor (Mallinckrodt Sensor Systems, Inc, Ann Arbor, Mich). Hemoglobin levels (in grams per deciliter) were measured from arterial blood samples (482 Co-Oximeter; Instrumentation Laboratory Corp, Lexington, Mass). Cerebral delivery of oxygen (CDO_2 in milliliters per 100 g brain per minute), cerebral metabolic rate of oxygen (CMRO_2 in milliliters per 100 g brain per minute) and cerebral oxygen extraction (CEO_2 as a percent) were calculated as follows:

$$\text{CDO}_2 = \text{CBF} \times \text{arterial oxygen content}$$

$$\text{CMRO}_2 = \text{CBF} \times (\text{arterial oxygen content} - \text{sagittal sinus venous oxygen content})$$

$$\text{CEO}_2 = (\text{CMRO}_2 / \text{CDO}_2) \times 100\%$$

The oxygen content (in milliliters of oxygen per milliliter of blood) was calculated by the following formula:

$$\text{Oxygen content} = 0.01 \times [(1.36) (\text{hemoglobin}) (\text{oxygen saturation}) + (0.003) (\text{oxygen tension})]$$

Experimental protocol and data collection. The animals were randomized into 2 groups with 6 animals in each group.

Table I. Global and regional CBF in control and PBN groups before and after DHCA

	Group	Pre-DHCA	Post-DHCA	P value
Global CBF	Control	48.4 ± 3.6	25.1 ± 2.4	.0001
	PBN	46.9 ± 3.0	37.2 ± 4.9	.01
Hemispheres	Control	50.1 ± 2.7	22.6 ± 1.2	.0003
	PBN	46.9 ± 2.5	31.0 ± 3.3	.003
Cerebellum	Control	55.2 ± 2.5	39.9 ± 3.0	.009
	PBN	50.4 ± 2.8	50.9 ± 2.8	.87
Basal ganglia	Control	47.1 ± 1.1	27.3 ± 1.3	.0001
	PBN	40.2 ± 3.5	34.7 ± 3.0	.19
Brain stem	Control	40.6 ± 2.3	26.1 ± 1.3	.001
	PBN	36.5 ± 2.1	36.1 ± 2.3	.89

The study group (PBN) received 100 mg · kg⁻¹ α-phenyl-*tert*-butyl nitron in 5 mL of normal saline solution intravenously at induction plus an additional 200 mg · kg⁻¹ in 10 mL of normal saline solution in the pump. Control animals received 5 mL of normal saline solution intravenously at induction plus 10 mL in the pump. All animals underwent 60 minutes of DHCA at 18°C and were rewarmed and reperfused for 1 hour. CBF measurements were all determined at a CPP of 50 mm Hg. All values are given in units of mL · 100 g⁻¹ · min⁻¹.

Table II. Global cerebral oxygen handling and CVR in control and PBN groups before and after DHCA

	Group	Pre-DHCA	Post-DHCA	P value
CDO ₂ (mL · 100 g ⁻¹ · min ⁻¹)	Control	6.15 ± 0.55	3.19 ± 0.30	.0003
	PBN	5.79 ± 0.43	4.97 ± 0.72	.11
CMRO ₂ (mL · 100 g ⁻¹ · min ⁻¹)	Control	3.53 ± 0.22	2.17 ± 0.20	.0002
	PBN	3.61 ± 0.29	3.60 ± 0.50	.98
CEO ₂ (%)	Control	58.3 ± 3.4	68.9 ± 4.3	.03
	PBN	62.9 ± 4.1	72.8 ± 3.0	.05
CVR (mm Hg · 100 g · min · mL ⁻¹)	Control	1.06 ± 0.08	2.03 ± 0.20	.001
	PBN	1.08 ± 0.07	1.48 ± 0.22	.05

The study group (PBN) received 100 mg · kg⁻¹ α-phenyl-*tert*-butyl nitron in 5 mL of normal saline solution intravenously at induction plus an additional 200 mg · kg⁻¹ in 10 mL of normal saline solution in the pump. Control animals received 5 mL of normal saline solution intravenously at induction plus 10 mL in the pump. All animals underwent 60 minutes of DHCA at 18°C and were rewarmed and reperfused for 1 hour. CDO₂, Cerebral oxygen delivery; CEO₂, cerebral oxygen extraction; CMRO₂, cerebral metabolic rate of oxygen; CVR, cerebral vascular resistance.

The study group received a PBN concentration of 100 mg · kg⁻¹ (Sigma Chemical Company, St Louis, Mo) in 5 mL of normal saline solution intravenously at induction plus an additional 200 mg · kg⁻¹ in 10 mL of normal saline solution in the pump. Control animals received 5 mL of normal saline solution intravenously at induction plus 10 mL in the pump. All animals were cannulated for CPB, and normothermic perfusion was commenced at 120 mL · kg⁻¹ · min⁻¹. The animals were stabilized on normothermic CPB for a minimum of 20 minutes before the pre-DHCA measurement was taken. During this time the pump flow was adjusted to provide a constant CPP of 50 mm Hg. After the pre-DHCA measurement, pump flow was returned to 120 mL · kg⁻¹ · min⁻¹ and the animals were cooled for DHCA. At the end of 60 minutes of DHCA, circulation was recommenced and the animals were rewarmed. At 45 minutes of reperfusion, pump flow rate was again adjusted to provide a CPP of 50 mm Hg for 15 minutes before the post-DHCA CBF measurement was made at 60 minutes of reperfusion. Data collected at the two time points included nasopharyngeal temperature, mean arterial blood pressure, right atrial pressure, sagittal sinus venous pressure, arterial and sagittal sinus blood gases, CPB flow

rate, electrolytes, hematocrit, hemoglobin, CBF, and renal blood flow.

Statistical analysis. All the results were entered into an Excel 97 spreadsheet (Microsoft Corporation, Redmond, Wash) for further analysis. Repeating formulas were set up to calculate the mean and standard error of the mean for all the data collected in each animal. Further repeating formulas were programmed for calculation of the CBF, CDO₂, CEO₂, CMRO₂, and the percentage change between these, before and after DHCA, for all animals. A 2-tailed paired samples *t* test was used to compare means at different time points within a group. An unpaired (independent samples) *t* test was used to compare means between the groups. Statistical significance was tested at the 95% confidence limit.

Results

Before the commencement of cooling and DHCA, there was no significant difference in global CBF, CVR, cerebral oxygen handling, and renal blood flow between the 2 groups (*P* > .21). The nasopharyngeal temperature, arterial blood gases, pH, hematocrit,

Fig 1. Global and regional CBF at 1 hour of reperfusion after 60 minutes of DHCA at 18°C in control and PBN groups. Data are expressed as percentage of baseline CBF before DHCA (mean $\pm$ standard error of the mean). *HEMI*, Cerebral hemispheres; *CBLM*, cerebellum; *BG*, basal ganglia; *BS*, brain stem. *Significantly greater percentage recovery than control group, unpaired t test $P < .02$. †Not significantly different from pre-DHCA value within group, paired t test $P > .19$.

Fig 2. Global and regional CMRO₂ at 1 hour of reperfusion after 60 minutes of DHCA at 18°C in control and PBN groups. Data are expressed as percentage of baseline CBF before DHCA (mean $\pm$ standard error of the mean). *HEMI*, Cerebral hemispheres; *CBLM*, cerebellum; *BG*, basal ganglia; *BS*, brain stem. *Significantly greater percentage recovery than control group, unpaired t test $P < .04$. †Not significantly different from pre-DHCA value within group, paired t test $P > .1$.

hemoglobin, and CPP were also similar in both groups ($P > .29$, data not shown). Within each group, no significant differences (paired samples t test $P > .1$) in these variables were detected before and after DHCA.

Effects of DHCA in the control group. In the control group, after 1 hour of DHCA the systemic vascular resistance fell from 0.43 ± 0.03 mm Hg $\cdot$ 100 g $\cdot$ min $\cdot$ mL⁻¹ before DHCA to 0.35 ± 0.02 mm Hg $\cdot$ 100 g $\cdot$ min $\cdot$ mL⁻¹ after DHCA ($P = .06$). This fall necessitated a rise in mean pump flow from 137 ± 8 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹ before DHCA to 170 ± 10 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹ after DHCA ($P = .04$) to maintain the preset CPP of 50 mm Hg at the time of CBF measurement. CBF was significantly reduced to all regions of the brain after DHCA ($P < .009$) (Table I). Different brain regions were affected to varying degrees, with recovery of blood

flow lowest in the cerebral hemispheres and greatest in the cerebellum (Fig 1).

Changes in global CDO₂, CMRO₂, CEO₂, and CVR after DHCA are shown in Table II. Global CDO₂ was significantly lower after 60 minutes of DHCA. Analysis of the regional CDO₂ demonstrated that, in accordance with CBF, there was regional variation with a significant difference in CDO₂ before and after DHCA in all regions ($P < .02$). Recovery in CDO₂ varied from only $45.9\% \pm 3.2\%$ of baseline in the cerebral hemispheres and $73.2\% \pm 5.6\%$ of the baseline in the cerebellum. The mean global CMRO₂ also dropped significantly after DHCA. The recovery in CMRO₂ after DHCA varied between the brain regions. The percentage recovery was highest in the cerebellum and lowest in the cerebral hemispheres (Fig 2). Both the CEO₂ and the CVR were significantly higher after DHCA.

Fig 3. Renal blood flow in the control and PBN groups at initial baseline (*Pre-DHCA*) and at 1 hour of reperfusion (*Post-DHCA*) after 60 minutes of DHCA at 18°C. Data are expressed as mean $\pm$ standard error of the mean. *Significant difference from pre-DHCA value within group, paired *t* test $P = .04$. †Significant difference from post-DHCA control value, unpaired *t* test $P = .03$.

Finally, in the control group the renal blood flow fell significantly after DHCA to $80.1\% \pm 6.2\%$ of the flow before DHCA (Fig 3).

Effects of PBN. In the PBN group the systemic vascular resistance was higher after DHCA than before DHCA (0.56 ± 0.04 mm Hg $\cdot$ 100 g $\cdot$ min $\cdot$ mL⁻¹ compared with 0.37 ± 0.02 mm Hg $\cdot$ 100 g $\cdot$ min $\cdot$ mL⁻¹, $P = .0005$ before DHCA) and the mean pump flow was lower (107 ± 7 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹ compared with 157 ± 8 mL $\cdot$ kg⁻¹ $\cdot$ min⁻¹ before DHCA, $P = .001$). The change in pump flow reflects maintenance of the preset level of CPP at 50 mm Hg despite changes in the systemic vascular resistance. The change in systemic vascular resistance and pump flow in the control and the PBN groups in opposite directions resulted in a highly significant difference between the 2 groups after DHCA ($P = .0004$).

Recovery of CBF after DHCA in the PBN group was significantly greater than in the control group globally and in all brain regions ($P < .02$) (Fig 1). Recovery of flow to the cerebellum, brain stem, and basal ganglia occurred to such an extent that there was *no* significant difference between the regional blood flow determined before and after DHCA in these regions. Global cerebral oxygen handling in the PBN group is shown in Fig 4. After DHCA there was no significant change in global CDO₂ (Table II). Regional analysis determined that only in the cerebral hemispheres, where CDO₂ fell from 5.8 ± 0.3 to 4.1 ± 0.4 mL $\cdot$ 100 g⁻¹ $\cdot$ min⁻¹, was there a significant difference in CDO₂ before and after DHCA ($P = .01$). In all regions, the percentage recovery of CDO₂ was significantly greater in the PBN group than in the control group ($P < .01$). After DHCA in the PBN group, recovery in CMRO₂ both globally and in

Fig 4. Cerebral oxygen handling in the PBN group at initial baseline (*Pre-DHCA*) and at 1 hour of reperfusion (*Post-DHCA*) after 60 minutes of DHCA at 18°C. Data are expressed as mean $\pm$ standard error of the mean. *Significant difference from pre-DHCA value within group, paired *t* test $P = .05$. †Significant difference from post-DHCA control value, unpaired *t* test $P < .05$.

all regions, occurred to such an extent that no significant difference was detected between the pre-DHCA and post-DHCA values ($P > .1$). Regional recovery of the CMRO₂ was significantly higher than in the control group in all brain regions ($P < .04$) (Fig 2).

The CVR was higher in the PBN group after DHCA than before (Table II). When expressed as a percentage of the baseline CVR before DHCA, however, the increase was significantly less in the PBN group than in the control group ($133.6\% \pm 12.3\%$ compared with $190.6\% \pm 7.1\%$, $P = .002$). The rise in CVR in the PBN group is therefore significantly less than in the control group. Finally, there was no significant difference between the mean renal blood flow before and after DHCA in the PBN group ($P = .08$) (Fig 3).

Discussion

The brain is probably the organ most susceptible to damage by oxygen-derived free radicals. One of the reasons is that the brain is particularly enriched in highly peroxidizable fatty acids. In addition, certain regions of the brain have a higher iron content than other tissues,¹⁵ which is important because the rapid generation of OH radicals from H₂O₂ during the Fenton reaction is dependent on iron (or copper) as a catalyst. Furthermore, the brain does not contain high levels of the protective enzymes superoxide dismutase, catalase, or glutathione peroxidase. Finally, the levels of ascorbate in the brain are high, which is relevant because iron can only catalyze the formation of OH radicals after it has been reduced by ascorbate from the ferric to ferrous forms. In all, this is a rather bad combination of factors that leads to promotion of

oxidative damage in the brain if disorganization of the tissue occurs.¹⁶

In the current study, after 60 minutes of DHCA at 18°C and 60 minutes of rewarming in the control group, the systemic vascular resistance fell and the pump flow was therefore increased to maintain a constant CPP at the time of CBF measurement. The CVR rose after DHCA, resulting in a fall in CBF. Production of free radicals during reperfusion may be responsible for this reduction in CBF. Damage by oxygen-derived free radicals during cerebral reperfusion has previously been shown to be important in the etiology of reduced CBF (and CMRO₂ abnormalities) after ischemia.¹⁷ It is also of interest that the production of lipid peroxides, which are generated by free radical damage, after subarachnoid hemorrhage is thought to be responsible for the subsequent cerebral vasospasm seen in this condition.¹⁸ As the arterial oxygen content was maintained at a constant level, the reduction in CBF was primarily responsible for the drop in CDO₂ seen in all brain regions after DHCA. Oxygen extraction was increased and the combination of reduced CBF and increased CEO₂ resulted in a fall in the CMRO₂ in all regions. The finding of impaired CMRO₂ is consistent with previous studies of normothermic global cerebral ischemia-reperfusion injury.¹⁷

In the animals that received PBN, the systemic vascular resistance rose significantly after DHCA. The increase in systemic vascular resistance in the DHCA group compared with the control group suggests that in the control group the drop in resistance was due to systemic vasodilatory effects of free radicals. After DHCA the CBF was significantly greater in the PBN group than in the control group in all brain regions. This suggests that the reduction of CBF in the control group was due, at least in part, to an increase in free radical activity as a result of DHCA. Previous experimental evidence has shown that PBN results in improvement in CBF after cerebral ischemia-reperfusion injury.^{19,20} A study by Inanami and Kuwabara¹⁹ showed that PBN increased CBF in a dose-dependent manner. Furthermore, inhibition of nitric oxide synthase, an enzyme that produces nitric oxide, resulted in attenuation of the PBN effect. This suggests that the PBN-induced increase in CBF is strongly associated with nitric oxide-related vasodilatation.¹⁹ Schulz and associates²⁰ suggested that the vascular endothelium was a primary target for the damaging action of free radicals.

Recovery of CDO₂ occurred to such an extent both globally and in all areas of the brain except the cerebral hemispheres that there was no significant difference before and after DHCA. This reflects the improvement

in post-DHCA CBF with PBN. After DHCA there was no significant difference in CMRO₂ compared with the pre-DHCA level. Therefore, when PBN is given before CPB, a normal cerebral metabolic recovery results after 60 minutes of DHCA. PBN results in a pronounced recovery of energy state after focal cerebral ischemia, with both adenosine triphosphate and lactate contents approaching normal.²¹ The improvement in metabolic recovery with PBN is thought to be due to the effect of the spin trap at the mitochondrial level.²²

PBN improves the histologic and neurobehavioral recovery in both focal and global models of cerebral ischemia. The size of the infarct is reduced after both permanent and transient middle cerebral artery occlusion.^{20,23} In addition, a reduction in cerebral edema, attenuation of neuronal damage in the CA1 area of the hippocampus, and improvement in neurologic behavior test results have been demonstrated with PBN after cerebral ischemic injury.²³⁻²⁵ In one study a significant improvement in neurobehavioral score was seen even when PBN was given 12 hours after the ischemic episode.²³ In combination, these findings demonstrate that PBN has a strong neuroprotective effect and support the hypothesis that free radicals play an important role in brain injury after cerebral ischemia.

In addition to the beneficial effects of PBN that have been demonstrated in models of cerebral ischemia, its use has been shown to provide protection for other organs including the heart,^{26,27} lungs,²⁸ and kidneys²⁹ in a variety of experimental models. In the current study, a significant improvement in renal blood flow was noted in the PBN group after DHCA. This effect has the potential to reduce renal morbidity after DHCA and could have important implications in pediatric cardiac surgical practice.

Free radicals are known to contribute to tissue injury in a wide variety of pathologic conditions including trauma, Parkinson disease, arthritis, carcinogenesis, ionizing radiation, ozone damage, and aging. In addition, there is now considerable evidence that oxygen-derived free radicals are responsible for cell damage in the course of cerebral ischemia. This study suggests that free radicals are involved in the impairment in cerebral recovery that follows a period of DHCA, and the null hypothesis stated previously can be rejected. The most likely mechanism of improved outcome after treatment with PBN in the current study is attenuation of free radical injury; however, other unknown effects of the drug cannot be completely excluded. This is the first study in the context of CPB to implicate free radicals in cerebral damage. A remarkable improvement in CBF and CMRO₂ after DHCA was demonstrated with

the use of PBN in the neonatal piglet. This suggests that free radical scavengers could have enormous potential as cerebral neuroprotectants in patients undergoing surgery with the use of DHCA.

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Discussion

Dr F. Mark Lupinetti (Seattle, Wash). Is it possible that conventional treatment fortuitously achieves the same benefits that PBN does? Your model does not include agents such as steroids, barbiturates, or mannitol, which are commonly used in clinical practice. Would the inclusion of these agents overcome the benefits of PBN?

Dr Langley. The main reason for not using other potentially neuroprotective strategies was that we wanted to maximize the injury caused by DHCA in the control group. You mentioned barbiturates, mannitol, and steroids, but there are a number of other mechanisms that we could also have eval-

uated. Dr Jonas' group in Boston has looked at aprotinin and *N*-methyl-D-aspartate antagonists, monoclonal antibodies to CD18, and our own group has investigated the beneficial effects with thromboxane A₂ antagonists and platelet activating factor antagonists. One of the problems of using a combination of strategies was determining which of the chemicals or compounds is producing the effect. We wanted to create a study that was very clear-cut, without any potentially confounding variables.

Dr Lupinetti. My second question is a little more philosophical. As we focus more carefully on brain protection, how can we avoid the unfortunate situation that currently exists in the field of myocardial protection? As you know, more than 1000 agents have been tested as possibly benefiting the ischemic heart and, remarkably enough, all of them work. This has generated numerous publications but relatively little patient benefit. I would appreciate your thoughts regarding how we might better compare competing strategies of cerebral protection and ultimately improve our clinical outcomes.

Dr Langley. That is a very interesting question and one that is difficult to answer. I am not entirely sure at the present time, however, whether an analogy between myocardial protection and cerebral protection is quite fair. Cerebral neuroprotection during cardiac surgery is fairly poorly understood. It is extremely complicated, and our comprehension of the mechanisms involved is still in its infancy. With respect to the research in the field of myocardial protection, I do not think we are talking here about adding a bit of magnesium or adding a bit of calcium to a cardioplegic solution. The current study demonstrates interruption of what is possibly the most important pathway leading to cerebral injury after ischemia. This study has demonstrated that free radicals are involved in the impairment of cerebral recovery and that a free radical scavenger, in this instance PBN, can protect the brain from free radical-mediated damage. I do not think it is necessary to investigate a whole battery of different free radical scavengers. The mechanism has been fairly convincingly demonstrated. We are eager to undertake one further animal experiment, a survival model in which we could evaluate neurobehavioral outcomes. After this, we are eager to move fairly rapidly into clinical applications of our findings.

Dr Vaughn Starnes (*Los Angeles, Calif*). I have two comments about how you did the study.

Do you know whether PBN has any vasodilatory effect itself? You controlled perfusion on the basis of pressure and not flow. Were the flow characteristics in the 2 groups different? In other words, did the PBN-treated group get 30% more blood flow because you were controlling pressure, not flow?

Dr Langley. We deliberately controlled CPP because it has previously been clearly demonstrated that cerebral perfusion is determined by perfusion pressure rather than by flow rate. The pump flow rate was actually significantly lower in the PBN group than in the control group.

Dr Starnes. Was there a flow difference in the 2 groups?

Dr Langley. Yes, there was. In the control group the systemic vascular resistance fell after DHCA. This fall necessitated a rise in pump flow rate to maintain the preset CPP of 50 mm Hg. In the PBN group the systemic vascular resistance was higher after DHCA and the pump flow rate was therefore reduced. The improved CBF in the PBN group can therefore not be attributed to an increase in pump flow rate.

Dr W. R. Eric Jamieson (*Vancouver, British Columbia, Canada*). Would you give some consideration to evaluating the two current clinical techniques—retrograde cerebral perfusion under profound hypothermia and direct arterial reperfusion under moderate hypothermia—and use the same model to assess these two current clinical parameters of management?

Dr Langley. Yes, we would. I think it would be very interesting to do that.

Dr Frank Pigula (*Pittsburgh, Pa*). Did you evaluate the metabolic state of the brain in any other way in terms of glucose metabolism or lactate production? Did you evaluate the potential for edema in the brain after DHCA?

Dr Langley. We did not evaluate lactate levels in this study. In the study that we presented to the Southern Thoracic Surgical Association in November 1998, which will be published in the *Annals of Thoracic Surgery*, we demonstrated a correlation between cerebral ultrastructure in the brain and CMRO₂. We found that an abnormal CMRO₂ was associated with perivascular and intracellular organelle edema and that when the CMRO₂ recovered to a normal level there was a remarkable recovery in the ultrastructural findings in the brain. We believe that the normal recovery of CMRO₂ in this study would be associated with a normal cerebral perivascular ultrastructural recovery.