Brain oxygen and metabolism during circulatory arrest with intermittent brief periods of low-flow cardiopulmonary bypass in newborn piglets

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Objective: We performed this study to determine whether brief intermittent periods of low-flow cardiopulmonary bypass during deep hypothermic circulatory arrest would improve cortical metabolic status and prolong the “safe” time of deep hypothermic circulatory arrest.

Methods: After a 2-hour baseline, newborn piglets were placed on cardiopulmonary bypass and cooled to 18°C. The animals were then subjected to 80 minutes of deep hypothermic circulatory arrest interrupted by 5-minute periods of low-flow cardiopulmonary bypass at either 20 mL · kg⁻¹ · min⁻¹ (LF-20) or 80 mL · kg⁻¹ · min⁻¹ (LF-80) during 20, 40, 60, and 80 minutes of deep hypothermic circulatory arrest. All animals were rewarmed, separated from cardiopulmonary bypass, and maintained for 2 hours (recovery). The oxygen pressure in the cerebral cortex was measured by the quenching of phosphorescence. The extracellular dopamine level in the striatum was determined by microdialysis. Results are means ± SD.

Results: Prebypass oxygen pressure in the cerebral cortex was 65 ± 7 mmHg. During the first 20 minutes of deep hypothermic circulatory arrest, cortical oxygen pressure decreased to 1.3 ± 0.4 mmHg. Four successive intermittent periods of LF-20 increased cortical oxygen pressure to 6.9 ± 1.2 mmHg, 6.6 ± 1.9 mmHg, 5.3 ± 1.6 mmHg, and 3.1 ± 1.2 mmHg. During the intermittent periods of LF-80, cortical oxygen pressure increased to 21.1 ± 5.3 mmHg, 20.6 ± 3.7 mmHg, 19.5 ± 3.95 mmHg, and 20.8 ± 5.5 mmHg. A significant increase in extracellular dopamine occurred after 45 minutes of deep hypothermic circulatory arrest alone, whereas in the groups of LF-20 and LF-80, the increase in dopamine did not occur until 52.5 and 60 minutes of deep hypothermic circulatory arrest, respectively.

Conclusions: The protective effect of intermittent periods of low-flow cardiopulmonary bypass during deep hypothermic circulatory arrest is dependent on the flow rate. We observed that a flow rate of 80 mL · kg⁻¹ · min⁻¹ improved brain oxygenation and prevented an increase in extracellular dopamine release.

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The duration of deep hypothermic circulatory arrest (DHCA) is thought to be a critical factor in the neuropsychological outcomes in infants and children when it is used in the repair of complex congenital heart defects. It is generally accepted that 30 to 40 minutes of DHCA at 18°C is a safe period, and if it is exceeded, according to experimental data and clinical experience, the risk of neuropsychological dysfunction increases. Different brain regions are selectively vulnerable to DHCA. Clinical evidence suggests that in the human infant, DHCA preferentially damages the basal ganglia, which control tone and movement. The main input site of the basal ganglia is the striatum, a highly dopaminergic region of the brain.
Consistent with clinical observations, animal studies have shown that prolonged DHCA can trigger biochemical alterations in the different regions of brain and can cause neuronal degeneration, cell death, or both. Kurth and associates\(^1\) reported and characterized regional distribution of cell death in the brain after DHCA in newborn piglets, presenting evidence that DHCA selectively damages neurons within the neocortex, hippocampus, and striatum. De-Leon and associates,\(^2\) in experiments on dogs, showed that profoundly hypothermic cardiopulmonary bypass (CPB) caused neuronal loss and degeneration within the cortex and caudate nucleus. Similarly, Tseng and colleagues\(^3\) showed in dogs that, after circulatory arrest, apoptosis occurred in selected neuronal populations, including the hippocampus, striatum, and neocortex. After cardiac arrest in 1- to 2-week-old piglets, necrosis was the dominant form of cell death, affecting the striatum earlier, more uniformly, and to a greater degree than other regions.\(^4\)

Because of concern regarding the effects of prolonged DHCA on brain oxygenation and cell injury, different techniques, such as CPB combined with low-flow or selective regional cerebral perfusion, have been investigated. The possible protective effects of these techniques on brain oxygenation and metabolism were addressed in our early studies.\(^5,6\) The purpose of this investigation was to assess whether intermittent brief periods of low-flow CPB (LF) during prolonged DHCA can increase cortical oxygenation and delay detrimental metabolic changes in the brain. By showing that changes in perfusion techniques can prolong the “safe period” of DHCA, we may be able to modify the perfusion approach and, consequently, improve the neuro-psychological outcome of the neonates and infants requiring congenital heart surgery.

In our model, we have used oxygen-dependent quenching of phosphorescence to continuously measure the oxygen levels within the microvasculature of the neocortex. This method directly measures the free oxygen within the blood plasma of the microcirculation within the neocortical tissue. In addition to assessing cortical brain oxygenation, we measured the changes in striatal extracellular levels of dopamine. The changes in dopamine have been shown to be essentially independent of blood flow and pH and therefore make it a very sensitive marker for adequate brain oxygenation.\(^7\) Dopamine itself might also be a mediator of neuronal injury, particularly at high levels within the striatum.

### Materials and Methods

#### Animal Model

Newborn piglets aged 3 to 5 days (1.4-2.5 kg) were anesthetized with halothane, and a tracheotomy was performed. The piglets were then placed on a ventilator, and anesthesia was maintained with fentanyl, isoflurane 0.5%, and pancuronium. Femoral venous and arterial cannulas were placed for the collection of blood samples and for monitoring blood pressure. With the head of the animal in a stereotaxic holder, the scalp was removed, and a cranial window approximately 8 mm in diameter was created over the right parietal hemisphere for measurement of cortical oxygenation. A small hole was drilled over the left parietal hemisphere for implantation of a microdialysis probe into the left striatum. After a 2-hour stabilization period, CPB was performed. After bypass, the animals were recovered for 2 hours and then killed with 4 mol/L KCl. All animal procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee.

#### CPB Technique

The circuit was primed with Plasmalyte-A (Baxter Healthcare, Deerfield, Ill), and then 25% albumin was added to the circuit. Donor whole blood was added to maintain a hematocrit of 25% to 30%. Heparin (1000 U), fentanyl (50 \(\mu\)g), pancuronium (1 mg), CaCl\(_2\) (500 mg), methylprednisolone (60 mg), cefazolin (100 mg), furosemide (2 mg), and NaHCO\(_3\) (25 mEq) were then added to the pump prime. A membrane oxygenator (Lilliput, Cobe Cardiovascular, Arvada, Colo) was used, as was a roller pump system (Cobe Cardiovascular) and arterial filter (Terumo Cardiovascular, Ann Arbor, Mich). For CPB, a median sternotomy was performed. Before cannulation, 500 U of heparin was administered intravenously. The ascending aorta was cannulated, as was the right atrial appendage. The full CPB flow rate was set at 150 mL · kg\(^{-1}\) · min\(^{-1}\), Alpha stat blood gas management was performed in all experiments.

#### Experimental Protocol

All animals were cooled to a nasopharyngeal temperature of 18°C over a 30-minute period. The piglets were randomly assigned to 1 of 3 groups. The first group (n = 8) had DHCA for 80 minutes. Groups 2 (n = 8) and 3 (n = 8) had four 20-minute periods of DHCA interrupted by 5-minute periods of LF at either 20 mL · kg\(^{-1}\) · min\(^{-1}\) (LF-20) or 80 mL · kg\(^{-1}\) · min\(^{-1}\) (LF-80), respectively. All animals were then rewarmed for 30 minutes, separated from CPB, and recovered for 120 minutes (Figure 1).

#### Measurements of Oxygen Pressure and Oxygen Distribution by the Oxygen-dependent Quenching of Phosphorescence

Cortical oxygen pressure was measured by using oxygen-dependent quenching of phosphorescence.\(^8-11\) The technical basis for determining the distribution of oxygen in the microcirculation of tissue from the distribution of phosphorescence lifetimes in the serum of blood has been described in detail.\(^12\) Briefly, a near-infrared oxygen-sensitive phosphor (Oxyphor G2, Oxygen Enterprises, Ltd., Philadelphia, Pa) was injected intravenously.
at approximately 1.5 mg/kg. The measurements were made by using a multifrequency phosphorescence lifetime instrument (PMOD 5000, Oxygen Enterprises, Ltd.) using algorithms and software developed by Vinogradov and coworkers. The excitation light (635 nm), modulated by the sum of 37 sinusoidal waves with frequencies spaced between 100 and 40 kHz, was carried to the tissue through a 3-mm light guide. The phosphorescence lifetime light guide. This positioning of the light guides allowed effective sampling of brain tissue oxygenation down to approximately 6 mm under the neocortical surface. The phosphorescence was optically filtered (3-mm-thick 695-nm-long pass Schott glass), and the signal from the detector was amplified, digitized, and analyzed to give the distribution of phosphorescence lifetimes (oxygen histogram) in the volume of tissue sampled by the light. Because there were substantial differences among animals with respect to collected phosphorescence and so on, the oxygen histograms were normalized to have the same total amount of signal (integral) for oxygen pressures less than 140 mm Hg.

Measurement of Striatal Extracellular Levels of Dopamine by Microdialysis

The dialysis probes have a molecular weight cutoff of 5 kd and a 300-μm outer diameter (Bioanalytical Systems Inc, West Lafayette, Ind). The implanted probes were continuously perfused at 1 μL/min with unbuffered Ringer solution with the following composition: 120 mmol/L NaCl, 2.5 mmol/L KCl, 1.3 mmol/L CaCl₂, and 0.9 mmol/L MgSO₄ (pH 7.0). After a 2-hour period of stabilization, the dialysis samples were collected at 15-minute intervals during the bypass and postbypass recovery. The perfusate samples were immediately analyzed for dopamine. The correct position of the dialysis probe was verified at the end of the experiments by sectioning of the brain and direct visualization.

Analysis of dopamine in the dialysates is performed on a BAS 200 (Bioanalytical Systems, Inc) liquid chromatography system. A BAS microbore octadecylsilane column (100 × 1 mm; 3-μm particle diameter) coupled with electrochemical detection was used to measure the dopamine. The dialysate (10 μL) was directly injected onto the microbore column. The detection limit under these conditions is 1 to 10 femtomoles per sample. Identification and quantitation of dopamine was conducted by comparison with chromatograms of a standard solution of dopamine. The efficiency of the microdialysis probe was determined in vitro at 18°C and 37°C for all of the compounds measured. The values for the levels of different compound in the dialysate are presented after correction for relative recovery by the microdialysis probe.

Statistical Analysis

All values are expressed as means ± SD for 8 experiments. Statistical significance was determined by using 1-way analysis of variance with repeated measures by the Wilcoxon signed rank test.

Results

Cortical Oxygen Pressure During DHCA and Intermittent LF in Newborn Piglets

The changes in cortical oxygen pressure during DHCA with and without intermittent LF are presented in Figure 2. During the first 20 minutes of DHCA, cortical oxygen pressure decreased to 1.3 ± 0.4 mm Hg. Four successive intermittent periods of LF-20 increased cortical oxygen pressure to 6.9 ± 1.2 mm Hg, 6.6 ± 1.9 mm Hg, 5.3 ± 1.6 mm Hg, and 3.1 ± 1.2 mm Hg. The subsequent DHCA periods after the LF-20 decreased the oxygen level exactly to the pre-LF level. When LF-80 was performed, 4 consecutive intermittent periods of LF-80 increased cortical oxygen pressure to 21.1 ± 5.3 mm Hg, 20.6 ± 3.7 mm Hg, 19.5 ± 3.95 mm Hg, and 20.8 ± 5.5 mm Hg. The oxygen pressure decreased during subsequent DHCA periods to values not significantly different from those of DHCA without LF.

Oxygen Distribution in Brain Tissue During DHCA and Intermittent LF in Newborn Piglets

Oxygen histograms for the cortex were measured at 15 minutes of each period of DHCA and at 4 minutes of each
intermittent low flow. Representative histograms for these experimental conditions are presented in Figure 3. As can be seen, the oxygen distribution is a bell-shaped curve. During control/prebypass conditions, the peak of the distribution was typically between 35 and 45 mm Hg, although it could be as high as 60 mm Hg. During DHCA at 18°C, the oxygen pressures decreased to 0 mm Hg, and the distribution became very narrow. When intermittent LF of 20 mL · kg⁻¹ · min⁻¹ was used, the oxygen pressures increased to peak values of nearly 10 mm Hg, and there was no longer any blood volume with oxygen pressures of 0 mm Hg. Thus, all of the vasculature in the tissue was perfused. Increasing the intermittent LF to 80 mL · kg⁻¹ · min⁻¹ further increased the oxygen pressures, with the peak value exceeding 20 mm Hg, and the distribution became quite broad.

Changes in Extracellular Striatal Dopamine During DHCA With Intermittent Periods of LF-20 and LF-80

The control value for extracellular dopamine was stable before CPB, with absolute concentrations of dopamine less than 1 pmol/mL. Figure 4 shows the observed changes in extracellular striatal dopamine during DHCA with and without intermittent periods of LF-20 and LF-80. The measured values are presented as a function of the time of DHCA; ie, the periods with low flow have been removed to allow direct comparisons of the time with no flow. As can be seen, a major, statistically significant increase in extracellular dopamine occurred after 45 minutes of DHCA alone compared with the groups of LF-20 and LF-80, in which the increase in dopamine did not occur until 52.5 and 60 minutes of DHCA, respectively.

Discussion

The purpose of this study was to determine whether brief intermittent periods of LF during DHCA could improve cortical metabolic status and prolong the safe time of DHCA. Our earlier studies showed that during DHCA, cortical oxygen pressure decreased rapidly to almost 0, and the excessive increase in the extracellular dopamine in the striatum occurred at 40 to 45 minutes of DHCA. The timing of this significant increase in extracellular dopamine correlates well with the generally accepted conclusion that, at 18°C, 30 to 45 minutes of DHCA results in no or questionably minimal brain injury. Periods of DHCA longer than 45 minutes may be associated with major changes in brain metabolism and a progressively increased risk of neuronal injury. This may, in turn, disrupt functional activity at selected synapses or cause neuronal damage and, perhaps, cell death.
On the basis of our earlier results, we examined whether intermittent brief periods of LF during prolonged DHCA improve outcome or allow a longer safe time of DHCA by providing periods of tissue oxygenation for metabolic recovery. We used an optical method, oxygen-dependent quenching of phosphorescence, to determine the distributions of oxygen within the cortex of the brain. These measurements are of the oxygen dissolved in the blood plasma in the microcirculation of the tissue and are the physiologically important oxygen source for cellular metabolism.

Our study reaffirms other experimental studies and clinical experience that at 45 minutes of DHCA, there is disruption of normal neuronal function characterized by a tremendous release of dopamine. We further have demonstrated that this release of dopamine is related to brain tissue hypoxia.

This study also showed that during the 5-minute periods of LF, the oxygen within the cortex increased significantly as compared with continued DHCA and that the level of oxygenation increased with increasing flow rate. At a flow of 20 mL · kg⁻¹ · min⁻¹, the increased level of brain tissue oxygen was small, with the peak of the oxygen histogram increasing to approximately 10 mm Hg. This oxygen pressure was around the P₅₀ level for oxygen binding to hemoglobin at 18°C, and this indicates that there was substantial extraction from the hemoglobin. More importantly, almost half of the microcirculation had oxygen pressures less than 10 mm Hg, and for much of the tissue this is not likely to be sufficient to supply the cellular oxygen requirements even at this low temperature. When the flow was increased to 80 mL · kg⁻¹ · min⁻¹, however, the oxygen pressures had much higher values; the peak increased to approximately 20 mm Hg. More importantly, the fraction of the blood plasma with oxygen pressures less than 10 mm Hg became very small, thus suggesting that most of the tissue was being provided with adequate amounts of oxygen. At 18°C, the role of the hemoglobin in oxygen delivery to tissue is greatly decreased relative to that at 37°C. This is in part because the oxygen affinity is much higher at these lower temperatures, so hemoglobin can not deliver oxygen except at low oxygen pressures, and in part because the solubility of oxygen in the blood plasma is increased enough to become a significant oxygen carrier. Ensuring adequate oxygen concentrations does not, however, ensure full metabolic function, and it is necessary to also evaluate metabolic function within the brain.

As a marker of brain metabolism in our experiments, we measured levels of extracellular dopamine within the striatal tissue. The extracellular level of dopamine in the striatum is an indicator of the exhaustion of cellular energy levels. The dopaminergic system of the striatum in a newborn piglet’s brain is very sensitive to hypoxia/ischemia insults, and even small decreases in oxygen pressure can cause statistically significant changes in both dopamine release/uptake and metabolism. In addition, the increase in extracellular dopamine can be a measure of the potential for cellular injury. Globus and colleagues and Filloux and Wamsley reported that unilateral infusion of 6-hydroxydopamine into the substantia nigra of rats to deplete dopamine before global ischemia resulted in significant protection of the dopamine-depleted striatum from ischemia-induced loss of medium-sized neurons. Marie and associates evaluated rat brain 72 hours after ischemia from a 4-vessel occlusion technique and reported that alpha-methyl-para-tyrosine treatment significantly decreased neuronal necrosis in the striatum but had no cytoprotective effect in the CA1 section of the hippocampus or in the neocortex. They suggested that the striatal cytoprotective effect of alpha-methyl-para-tyrosine is linked to cerebral dopamine depletion and that excessive dopamine release during ischemia plays a detrimental role in the development of ischemic cell damage in the striatum.

Dopamine can potentiate neuronal damage through several mechanisms, such as its effects on the glutaminergic system or increased production of free radicals. High levels of dopamine, iron, and oxygen are mostly responsible for the generation of free radicals, particularly in regions of the brain such as the putamen and the caudate nucleus.

One of the mechanisms of neuronal cell death after CPB and DHCA seems to be the formation of free radicals. Our early studies show that DHCA increases the level of o-tyrosine within the striatum of newborn piglets, thus indicating increased generation of hydroxyl radicals within the tissue. Free radicals are probably the major cause of both endothelial damage and brain edema after DHCA.

The significant increase in the levels of extracellular dopamine within the striatum appeared at a later time in our experiment when prolonged DHCA alone was compared with the 2 groups of LF. When the flow during these 5-minute periods was 80 mL · kg⁻¹ · min⁻¹, statistically significant increases in dopamine occurred 15 minutes later than with DHCA alone. A delay in dopamine release can be an indicator of a delay in changes in brain metabolism and neuronal injury, particularly within the striatum, and suggest added neuroprotection with this technique.

In conclusion, in this study performed on newborn piglets, interrupting DHCA with periods of low flow can prolong the safe period of DHCA. This safe time is dependent on the rate flow during intermittent LF. Using a flow of 80
mL ⋅ kg⁻¹ ⋅ min⁻¹ is sufficient to increase the oxygen pressures throughout the cortex to more than 20 mm Hg and seems to confer a neuroprotective effect of 15 minutes during prolonged DHCA. The experiments were performed in an animal model, newborn piglets, for DHCA. Clinical studies will need to be performed to validate our findings in neonates and infants.

References