Recovery of Evoked Potential Amplitude after Cerebral Arterial Air Embolism in the Rabbit

A Comparison of the Effect of Cardiopulmonary Bypass with Normal Circulation

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Background: Cerebral arterial air embolism (CAA) may cause neurologic injury during cardiac surgery. It is not known whether cardiopulmonary bypass (CPB) increases or decreases brain injury from CAA compared with the normal circulation.

Methods: A model of CAA was produced by injection of 50 μL/kg air into the internal carotid artery of methohexital-anesthetized New Zealand white rabbits. Somatosensory-evoked potential (SSEP) amplitude was measured serially as a marker of neurologic recovery. In experiment A, saline rather than air was injected to control for surgical manipulation and time in CPB (n = 4) and nonheparinized non-CPB (n = 4) animals. In experiment B, 50 μL/kg air was injected in CPB (n = 11) and nonheparinized non-CPB (n = 11) animals. In experiment C, non-CPB animals (n = 6) were given heparin according to the same protocol as for CPB.

Results: In experiment A, SSEP latencies and amplitudes did not differ between CPB and non-CPB conditions. In experiment B, there was no SSEP recovery 5 min after CAA in either CPB or non-CPB animals. Thereafter, SSEP recovery was less in CPB animals than in non-CPB animals at 30 min (9 ± 12% vs. 29 ± 20%: P = 0.009) and 60 min (18 ± 15% vs. 39 ± 22%: P = 0.030) after CAA. Ninety-minute SSEP recovery did not differ between CPB and non-CPB groups (at 24 ± 19% vs. 39 ± 24%, respectively; P = 0.146). In experiment C (heparinized non-CPB), SSEP recovery 5, 30, 60, and 90 min after CAA was 67 ± 48%, 72 ± 47%, 80 ± 35%, and 77 ± 35%, respectively.

Conclusions: Somatosensory-evoked potential recovery after CAA is no better (and is probably worse) during CPB than during normal circulation. The adverse effect of CPB occurs despite heparinization, which, under non-CPB conditions, appears to be protective. Therapies in addition to heparin are needed during CPB to reduce neurologic injury from CAA. (Key words: Brain, cardiac surgery; heparin.)

Although neurologic and neuropsychologic dysfunction is common after cardiac surgery, pathophysiologic mechanisms have not been fully elucidated.1–3 Pugsley et al.4 showed that microbubbles produced by bubble oxygenators result in a dose-dependent deterioration of postoperative neuropsychologic performance. Use of an arterial line filter decreased the number of cerebral arterial air emboli (CAA) detected by transcranial Doppler and improved neuropsychologic outcome.4 Thus, despite their small size (50–200 μm in diameter5,6), CAA from cardiopulmonary bypass (CPB) circuits cause clinically detectable brain injury. During cardiac surgery, CAA also originate from the operative field. Despite de-airing techniques, both micro- and macroscopic air is present in the left ventricle, the ascending aorta, or both in nearly all patients who have open-chamber procedures.7–9 Even in closed-chamber procedures, 15–50% of patients have substantial amounts of intracavitary air.7,9 With removal of the aortic cross-clamp, resumption of ejection, or both, intra-cardiac air enters the cerebral circulation. Whenever air is detected in the heart or aorta by transesophageal echocardiography, CAA are detected by transcranial
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Doppler. Given these collective observations, CAAE may be responsible for an important portion of the perioperative neurologic injury that occurs during cardiac surgery.

Our laboratory developed a model of CAAE in the rabbit. Previously we showed that, during non-CBP conditions, neurologic impairment from CAAE is (1) dose-dependent and (2) inversely correlated with recovery of somatosensory-evoked potential (SSEP) amplitude (i.e., lesser SSEP recovery is associated with greater neurologic impairment). The conditions of CPT might influence the effect of CAAE on the brain. For example, during CPB, hyperglycemia, marked anemia, and increased inflammatory activity (e.g., complement and neutrophil activation) could exacerbate brain injury from CAAE. The latter condition could be especially detrimental because several studies indicate that neurologic injury from CAAE is related to rapidly acting inflammatory processes. On the other hand, CPB could result in less brain injury from CAAE because of the presence of heparin. In addition to inhibiting thrombosis, heparin possesses a wide range of anti-inflammatory properties. In non-CPB studies, we found that heparin significantly decreases neurologic impairment from CAAE. Thus, to learn whether CPB favorably or unfavorably affects the brain’s response to CAAE, we compared SSEP recovery after CAAE under both CPB and non-CBP conditions in our rabbit model.

Materials and Methods

Experimental protocols were approved by the Animal Care and Use Committee of the University of Iowa in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 85-23, revised 1985). This study was conducted in three parts. In experiment A, we compared the stability of the SSEP signal in the presence of a placebo (saline) injection into the internal carotid artery, during CPB and normal (nonheparinized non-CPB) circulation. Therefore, in experiment A, saline, rather than air, was injected into the internal carotid artery. In experiment B, we compared SSEP recovery after intracarotid injection of 50 μl/kg air during CPB and nonheparinized non-CPB conditions. In experiment C, we measured SSEP recovery after injection of intracarotid air (50 μl/kg) in non-CPB animals that received heparin.

Basic Preparation

Anesthesia was induced in nonfasted New Zealand white rabbits of both sexes (weight, 3.7–5.1 kg) by inhalation of 5% isoflurane in oxygen. After cannulation of an ear vein and local infiltration with 1% lidocaine, a tracheotomy was performed and the trachea was intubated with a 3-mm cuffed endotracheal tube. On placement of the tracheal tube, animals were briefly paralyzed with a single dose of succinylcholine (1 mg/kg given intravenously). Thereafter, no paralytics were used. Animals were ventilated with 2% isoflurane in 30% oxygen - balance nitrogen to achieve normocarbia, monitored continuously with a calibrated anesthetic agent analyzer (Datex; Puritan-Bennett, Helsinki, Finland). Normal saline was infused intravenously at 4 ml·kg⁻¹·h⁻¹. Rectal temperature was maintained at 37–38°C with a servo-controlled heating pad.

Animals were placed prone in a stereotaxic frame (Kopf Instruments, Tuganda, CA) and the scalp was shaved. After skin incision, a 2-mm burr hole was drilled over the left frontoparietal cortex to expose the dura. A 1-mm thermocouple (K-type, L-08419-02; Cole Parmer, Chicago, IL) was placed between the cranium and dura to monitor epidural temperature. The bone defect was filled with bone wax. Stainless steel screws for recording SSEP were placed into the skull with the active electrode located over the left parietal region, 6 mm lateral to the midline and 1 mm anterior to the coronal suture. The reference electrode was placed midline into the maxillary bone.

Animals were turned supine and SSEP-stimulating needle electrodes were placed subcutaneously over the right median nerve. Through the left femoral artery, a saline-filled polyethylene catheter (PE-90; Intramedic, Parsippany, NJ) was advanced into the abdominal aorta for arterial pressure monitoring and intermittent blood sampling. The tip of a saline-filled catheter (PE-90), introduced via the right external jugular vein, was advanced to the superior vena cava to measure central venous pressure. Through a midline neck incision, the left external, internal, and common carotid arteries were isolated, and a branch of the external carotid (usually the facial) was selected for cannulation. Other branches of the external carotid were ligated with 4-0 silk and all bleeding points were cauterized. A saline-filled PE-50 catheter was introduced retrograde through the facial branch of the external carotid artery for later placement into the internal carotid artery (see below). The wound was covered with saline-soaked gauze until the time of air embolism.

Preparation for Cardiopulmonary Bypass

In animals assigned to receive CPB, an incision was made inferior to the left clavicle and the underlying
subclavian artery was isolated. The sternum was divided in midline, the thymus was retracted, and a pledgeted 4-0 silk purse-string suture was placed in the right atrium. After systemic anticoagulation with heparin (300 IU/kg given intravenously), the distal left subclavian artery was ligated and the vessel cannulated with a 2-mm pediatric arterial perfusion cannula (Stöckert Instrumente, Munich, Germany). A 21-French venous cannula (Polygan, Ballerup, Denmark) was placed in the right atrium. The aortic and right atrial cannulas were connected to the perfusion circuit, and CPB was initiated as described below. None of the steps described in this paragraph were performed on animals not undergoing CPB (non-CPB). Thus, in experiments A and B, non-CPB animals were not heparinized.

**Methohexital Anesthesia and Free Concentration Determination**

After isolation of the carotid system in non-CPB animals and after placement of the right atrial pursestring in CPB animals, normal saline and isoflurane were discontinued. Methohexital was administered as a bolus of 10 mg/kg given intravenously, followed by a continuous intravenous infusion (2 mg/ml in saline) to give 15 mg·kg⁻¹·h⁻¹. Methohexital was administered throughout the rest of the experiment (including CPB) to avoid isoflurane inhibition of SSEP. As will be described, methohexital administration was altered in CPB animals in an attempt to maintain similar free (unbound) methohexital concentrations in CPB and non-CPB animals.

At defined intervals (see below), 7-ml arterial blood samples were collected, chilled on ice, and centrifuged. Plasma samples were stored at −70°C until free methohexital assays were commercially performed (National Medical Services, Willow Grove, PA). Thawed plasma was centrifuged (15 min at 3,500 rpm) through a micropartition filter with a 30,000 molecular weight cutoff (Amicon Centrifree Micropartition System, Beverly, MA). The resulting aqueous phase is protein-free. To this, 20 μg/ml methylenephobarbital was added as an internal standard, followed by saturated ammonium chloride. Barbiturates were extracted into 5% isopropanol in methylene chloride. Barbiturates were separated, and concentrations were determined by gas chromatography using a 0.15-μm DB-17 column (J & W Scientific, Folsom, CA) using a carrier gas, a nitrogen phosphorus detector, and peak integration. The limit of quantitation is 0.02 μg/ml. The assay coefficient of variation equaled 8.7% at a free methohexital concentration of 0.5 μg/ml.

**Conduct of Cardiopulmonary Bypass**

The CPB circuit consisted of a venous reservoir, a membrane oxygenator/heat exchanger (Capiox 308, Terumo, Piscataway, NJ), a variable-temperature water pump (VWR Scientific, San Francisco, CA), and a centrifugal pump (BP-50 pump head, Biomedicus, Eden Prairie, MN). Circuit priming fluid consisted of 300 ml 6.5% (weight/volume) high molecular weight hydroxyethyl starch (McGaw Inc., Irvine, CA) in 0.72 N sodium chloride, 18 mEq sodium bicarbonate, 250 mg CaCl₂, and 1,000 IU heparin. Priming fluid was circulated through a 40-μm filter for 15–20 min before addition of ~150 ml filtered, packed rabbit erythrocytes, achieving a circuit hemoglobin concentration of 6–10 g/dl (OSM3; rabbit absorption coefficients; Radiometer, Copenhagen, Denmark).

Cardiopulmonary bypass was initiated and maintained at a systemic flow rate of 100 ml·kg⁻¹·min⁻¹, monitored with a calibrated in-line electromagnetic flow meter (TX-40P; Biomedicus). Water bath temperature to the oxygenator–heater exchanger was set at 38–39°C to maintain epidural temperatures of 37.5–38.5°C. The pulmonary artery was clamped to ensure complete venous outflow to the CPB circuit. To prevent left ventricular ejection, distention, or both, the tip of a 14-gauge catheter was placed transapically in the left ventricle to permit drainage to the venous reservoir. The oxygenator was ventilated with a variable mixture of oxygen and nitrogen to maintain partial arterial pressure of carbon dioxide (Pₐₘₜ) near 40 mmHg and partial arterial oxygen pressure (Pₐₒ₂) near 250 mmHg when measured at an electrode temperature of 37°C (IL1304; Instrumentation Laboratory, Lexington, MA). Blood from the surgical field was returned to the venous reservoir after passing through a 40-μm filter. Sodium bicarbonate was given to maintain a base excess greater than −4 mEq/l. Packaged rabbit erythrocytes were given to maintain arterial hemoglobin concentration between 7–8 g/dl during CPB. Supplemental heparin (100 IU/kg) was given intravenously at 30 and 60 min of CPB. No pharmacologic or mechanical means were used to control arterial pressure at any point in the experiment.

**Cerebral Arterial Air Embolism**

After a 15–20 min equilibration period, baseline (preembolism) physiologic measurements were obtained, including epidural temperature, mean arterial pressure, central venous pressure, arterial pH, Pₐₘₜ, Pₐₒ₂, hemoglobin concentration, sodium and potassium concentrations (Ciba Corning Diagnostics, Medfield, MA), and glu-
cose concentration (model 27, Yellow Springs Instrument Co., Yellow Springs, OH). At this time, baseline SSEP measurements were also obtained. Thereafter, a temporary aneurysm clip was placed across the left common carotid just proximal to its bifurcation. The facial artery catheter was directed into the proximal 1-2 mm of the internal carotid. Care was taken to avoid air entrapment in either the internal or external carotid arteries. Either 50 μl/kg saline (experiment A) or air (experiments B and C) was injected into the internal carotid, followed by 0.5 ml normal saline at a constant rate of 3 μl/s by infusion pump. Immediately thereafter, the aneurysm clip was removed from the common carotid and the injection catheter was withdrawn back into the external carotid, reestablishing between the internal and common carotid arteries. SSEPs and physiologic data were recorded at defined intervals after completion of air (or saline) injection.

After the last experimental SSEP was recorded, the injection catheter was reintroduced into the internal carotid artery and 200 μl/kg air was injected manually. This volume of air uniformly resulted in a total abolition of the SSEP signal and was given to confirm proper identification of the internal carotid artery. This occurred in all animals in all experiments. After demonstrating loss of SSEP, animals were killed by intracardiac injection of saturated potassium chloride solution.

Somatosensory-evoked Potentials

In previous studies with this model, we have found good correlations between postembolism SSEP amplitude and 4-h neurologic status and 24-h survival. Thus, in these experiments, recovery of SSEP amplitude was the primary outcome measure. As done in the previous studies, supramaximal square-wave DC pulses of 0.25-ms duration (four times motor threshold) were delivered at 1.4 Hz to the right median nerve. Sixty-four cortical responses were averaged with Grass model 10 evoked response system with bandpass filters of 0.3 and 10,000 Hz (Grass Instruments, Quincy, MA). High-amplitude electrical artifact was automatically rejected. The analog signal was converted to digital data by an A-D board interfaced with an IBM AT computer (Armonk, NY) for subsequent analysis. The amplitude of the primary cortical deflection was measured from the trough of the first major negative deflection (N1, occurring at ~13 ms) to the peak of the next positive deflection (P1, occurring at ~30 ms). N1 and P1 latencies were recorded for each animal before embolism; postembolism amplitude measurements were made from those points. The amplitude of the N1-P1 complex was expressed as a percentage of the baseline value for each animal.

Experiment A (Saline Injection: Cardiopulmonary Bypass vs. non-Cardiopulmonary Bypass)

The purpose of this experiment was to establish the time dependence of SSEP amplitude in the presence of surgical manipulation, cannulation, and saline injection into the internal carotid artery under CPB and non-CPB conditions. Thus, in experiment A, instead of air, 50 μl/kg saline was injected into the left internal carotid artery, as described before. Animals were randomly preassigned to undergo either CPB (group A1, n = 4) or not to undergo CPB (group A2, n = 4) during and after the saline injection, and were prepared as described above. Non-CPB animals were not heparinized. Somatosensory evoked potentials were recorded 5, 15, 30, 45, 60, and 90 min after intracarotid saline (and flush) injection. Physiologic and pharmacologic parameters were recorded at baseline and 45 and 90 min after saline injection.

To establish the effect of CPB on physiologically active methohexital concentration, we sampled arterial blood to determine free (unbound) methohexital concentration at baseline and 45 and 90 min after intracarotid saline injection. All non-CPB animals received a 10 mg/kg loading dose of methohexital followed by a 15 mg·kg⁻¹·min⁻¹ infusion. The first three CPB animals also received methohexital by this schedule. When CPB animals were found to have an approximately twofold greater free methohexital concentration than non-CPB animals (see Results section), one of four CPB animals received a 10 mg/kg loading dose and 7.5 mg·kg⁻¹·min⁻¹ infusion.

Experiment B (Air Embolism: Cardiopulmonary Bypass vs. non-Cardiopulmonary Bypass)

In experiment A, we established that there is no apparent difference between CPB and non-CPB animals in the stability of SSEP latencies or amplitude over time (see Results section). Therefore, the purpose of experiment B was to determine whether recovery of SSEP amplitude after CAAE differed between CPB and non-CPB conditions. A dose of 50 μl/kg air was chosen because, in a previous (non-CPB) study with this model, this dose was associated with ~50% recovery of SSEP amplitude and unequivocal neurologic impairment. Animals were randomly preassigned to undergo either CPB (group B1, n = 11) or not to undergo CPB (group...
B2, n = 11) during and after CAAE. Non-CPB animals were not given heparin. Based on free methohexital concentrations determined in experiment A (see Results section), CPB animals (group B1) received a methohexital loading dose of 10 mg/kg and an infusion rate of 10 mg·kg⁻¹·h⁻¹. Non-CPB animals (group B2) received a 10 mg/kg loading dose and 15 mg·kg⁻¹·min⁻¹ methohexital infusion. In CPB animals (B1), baseline (pre-CAAЕ) SSEP and physiologic measurements were obtained after 19 ± 6 min of CPB. The SSEPs were recorded 5, 15, 30, 45, 60, and 90 min after air (and flush) injection. Physiologic and pharmacologic parameters were recorded at baseline and 45 and 90 min after air injection.

Experiment C (Air Embolism: Heparinized Noncardiopulmonary Bypass)

The purpose of experiment C was to gauge the extent to which heparin might increase SSEP recovery. Group C (n = 6) was a non-CPB group identical to group B2, except that heparin was given as described for CPB animals (300 IU/kg intravenous bolus 30 min before CAAE with 100 IU/kg supplements 30 and 60 min after air embolism). In all animals, SSEPs were recorded 5, 15, 30, 45, 60, 75, and 90 min after injection of air (and flush), and arterial blood was collected for free methohexital determination at baseline and 90 min after CAAE.

Statistical Analysis

Data are expressed as means ± SD. Systemic physiologic and pharmacologic variables were assessed qualitatively. In experiment A, stability of SSEP amplitude over time was assessed in each group by calculating the slope of the relation between pooled SSEP amplitudes and time using linear least-squares regression. Confidence intervals were calculated by standard parametric techniques.

In experiment B, SSEP amplitudes 30, 60, and 90 min after CAAE were compared between groups using the two-sided Wilcoxon-Mann-Whitney test. Probability values were calculated using exact methods (StatXact 3 for Windows; Cytel Software Corp., Cambridge, MA). We chose to use a nonparametric method because SSEP data were skewed and had large differences in standard deviations among groups. In experiment B, potentially significant covariates included glucose, $P_{O2}$, and mean arterial pressure. For exploratory purposes, we used graphical methods (residuals vs. potential covariates and partial residual plots) and forward stepwise analysis of covariance to assess the effect of all measured physiologic variables on SSEP recovery (SYSTAT 5.03, Evanston, IL). However, because of multicollinearity, no reliable or significant pattern emerged.

Results

Experiment A (Saline Injection)

Table 1 summarizes physiologic and pharmacologic variables for experiment A. Cardiopulmonary bypass animals receiving maintenance infusions of methohexital at 15 mg·kg⁻¹·min⁻¹ (n = 3) had free methohexital concentrations that were two times greater than non-CPB animals maintained at the same rate (~2 µg/ml vs. ~1 µg/ml, respectively). In one CPB animal, the methohexital infusion was reduced to 7.5 mg·kg⁻¹·h⁻¹. This resulted in free methohexital concentrations slightly less than those in non-CPB animals. Based on these results, we predicted that a maintenance infusion of 10 mg·kg⁻¹·h⁻¹ would yield equivalent free methohexital concentrations (1-2 µg/ml) in CPB and non-CPB groups in the subsequent experiment (experiment B). Animal and human studies indicate that free methohexital concentrations in this range provide adequate anesthesia.²¹²² No animal demonstrated purposeful movement during the experiment, which was performed without paralysis.

$N_1$ (15 ± 1 ms) and $P_1$ (50 ± 5 ms) latencies did not differ between groups nor over time. Figure 1 shows SSEP amplitudes over time. Using pooled data over all time points, post-saline embolus SSEP amplitude did not differ from baseline in either CPB or non-CPB animals, at 104 ± 18% and 95 ± 23%, respectively. The SSEP amplitude did not significantly change over time in either the CPB group (slope = 0.17 ± 0.23% per minute [95% CI, −0.19 to 0.54]) or the non-CPB group (slope = 0.16 ± 0.46% per minute [95% CI, −0.57 to 0.89]). Therefore, there was no evidence of a time-related change in SSEP latency or amplitude during CPB or non-CPB conditions.

Experiment B (Air Embolism)

Table 2 summarizes physiologic and pharmacologic variables for experiment B. The CPB animals appeared to differ from non-CPB animals with respect to $P_{O2}$, and concentrations of glucose, hemoglobin, and methohexital (see Discussion).

Figure 2 shows SSEP amplitudes over time in CPB and non-CPB animals. In all animals, CAAE resulted in
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Table 1. Systemic Variables: Experiment A (Saline Injection)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>0 (baseline)</th>
<th>45 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A1: CPB</td>
<td>37.1 ± 0.5</td>
<td>37.3 ± 0.3</td>
<td>37.5 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>37.9 ± 0.7</td>
<td>37.5 ± 0.8</td>
<td>37.2 ± 0.9</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>A1: CPB</td>
<td>66 ± 9</td>
<td>75 ± 9</td>
<td>75 ± 12</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>96 ± 7</td>
<td>95 ± 11</td>
<td>83 ± 14</td>
</tr>
<tr>
<td>Central venous pressure (mmHg)</td>
<td>A1: CPB</td>
<td>4 ± 4</td>
<td>4 ± 3</td>
<td>4 ± 3</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>5 ± 3</td>
<td>5 ± 3</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>pH</td>
<td>A1: CPB</td>
<td>7.43 ± 0.03</td>
<td>7.38 ± 0.03</td>
<td>7.38 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>7.46 ± 0.09</td>
<td>7.41 ± 0.02</td>
<td>7.37 ± 0.03</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>A1: CPB</td>
<td>38 ± 5</td>
<td>40 ± 2</td>
<td>41 ± 1</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>38 ± 3</td>
<td>40 ± 3</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>A1: CPB</td>
<td>114 ± 18</td>
<td>227 ± 115</td>
<td>228 ± 65</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>192 ± 40</td>
<td>207 ± 29</td>
<td>199 ± 39</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>A1: CPB</td>
<td>9.2 ± 0.4</td>
<td>8.3 ± 0.6</td>
<td>8.0 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>11.6 ± 1.1</td>
<td>11.3 ± 0.8</td>
<td>11.0 ± 1.5</td>
</tr>
<tr>
<td>Na⁺ (mEq)</td>
<td>A1: CPB</td>
<td>145 ± 4</td>
<td>147 ± 3</td>
<td>149 ± 5</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>141 ± 5</td>
<td>144 ± 3</td>
<td>144 ± 2</td>
</tr>
<tr>
<td>K⁺ (mEq)</td>
<td>A1: CPB</td>
<td>3.7 ± 0.7</td>
<td>3.6 ± 0.6</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>3.0 ± 0.9</td>
<td>3.4 ± 0.5</td>
<td>3.4 ± 0.4</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>A1: CPB</td>
<td>171 ± 59</td>
<td>194 ± 89</td>
<td>181 ± 66</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>161 ± 42</td>
<td>140 ± 40</td>
<td>150 ± 44</td>
</tr>
<tr>
<td>Free methohexital (µg/ml)*</td>
<td>A1: CPB</td>
<td>1.5 ± 0.5 [0.5]</td>
<td>2.1 ± 0.6 [0.6]</td>
<td>2.3 ± 0.2 [0.5]</td>
</tr>
<tr>
<td></td>
<td>A2: non-CPB</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n = 4 in each group except for free methohexital in group A1 (CPB), where n = 3.
* Bracketed value is from one animal in which the methohexital infusion was halved (see text for explanation).

complete and instantaneous loss of SSEP signal. Five minutes after CAAE, SSEP amplitude had recovered to 1 ± 2% (median 0) in the CPB group and to 0 ± 2% (median 0) in the non-CPB group.§ Thereafter, there was a gradual partial recovery of SSEP amplitude over time. Thirty minutes after CAAE, SSEP recovery was 9 ± 12% (median 0%) in CPB animals compared with 29 ± 20% (median 29%) in non-CPB animals (P = 0.0086). At 60 min, SSEP recovery was 18 ± 15% (median 20%) in CPB animals compared with 39 ± 22% (median 47%) in non-CPB animals (P = 0.0292). At 90 min, SSEP recovery was 24 ± 19% (median 20%) in CPB animals compared with 39 ± 24% (median 43%) in non-CPB animals (P = 0.146).

Experiment C (Heparinized Noncardiopulmonary Bypass)

Table 3 summarizes physiologic and pharmacologic variables for heparinized non-CPB rabbits (group C). Figure 3 shows SSEP amplitudes over time. As before, CAAE resulted in complete and instantaneous loss of SSEP signal in all animals. However, by 5 min after CAAE, SSEP amplitude had recovered to 67 ± 48% (median 67%) of baseline. There was little additional SSEP recovery thereafter. The SSEP recovery at 30, 60, and 90 min in group C was 72 ± 47% (median, 64%), 80 ± 35% (median, 91%), and 77 ± 35% (median, 82%), respectively.

Discussion

Our results show that SSEP recovery after CAAE is certainly not better under CPB conditions than non-CPB conditions. In the first hour after CAAE, SSEP recovery was significantly worse with CPB. At 90 min after CAAE, differences in SSEP recovery between CPB and non-CPB

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§ This time refers to that following intracarotid injection of 50 µl/kg air and subsequently 500 µl saline flush. Because the infusion pump is set at 3 µl/s, the total time to deliver both air and flush is approximately 4 min. Thus the 5-min time point occurs approximately 9 min after the start of CAAE.
Table 2. Systemic Variables: Experiment B (Air Embolism)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Time after Air Embolism*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 (baseline)</td>
</tr>
<tr>
<td>Epidural temperature (°C)</td>
<td>B1: CPB</td>
<td>37.9 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>38.3 ± 0.4</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>B1: CPB</td>
<td>67 ± 11</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>83 ± 7</td>
</tr>
<tr>
<td>Central venous pressure (mmHg)</td>
<td>B1: CPB</td>
<td>2 ± 3</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>pH</td>
<td>B1: CPB</td>
<td>7.39 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>7.44 ± 0.03</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>B1: CPB</td>
<td>38 ± 2</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>B1: CPB</td>
<td>191 ± 67</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>195 ± 40</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>B1: CPB</td>
<td>7.7 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>12.3 ± 1.3</td>
</tr>
<tr>
<td>Na⁺ (mm)</td>
<td>B1: CPB</td>
<td>145 ± 3</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>142 ± 3</td>
</tr>
<tr>
<td>K⁺ (mm)</td>
<td>B1: CPB</td>
<td>3.7 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>B1: CPB</td>
<td>167 ± 60</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>117 ± 35</td>
</tr>
<tr>
<td>Free methohexital (μg/ml)</td>
<td>B1: CPB</td>
<td>1.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>B2: non-CPB</td>
<td>1.1 ± 0.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n = 11 in each group.
* In CPB animals, 0, 45, and 90 min after air embolism correspond to CPB durations of 19 ± 6, 77 ± 8, and 123 ± 8 min, respectively.

Groups did not achieve statistical significance, although mean recovery in CPB animals was still only half that of non-CPB animals. Failure to achieve statistical significance at 90 min was possibly due to small group sizes relative to the variance within each group. Previously we observed large variances among animals in SSEP recovery after CAAE. Despite this variance, there is a good correlation between SSEP recovery and subsequent neurologic status. Thus, on balance, the overall effect of CPB is to adversely affect SSEP recovery after CAAE.

Differences between Cardiopulmonary Bypass and Noncardiopulmonary Bypass Conditions

Animals that underwent CPB differed from those that did not in several respects. These differences typify alterations in systemic physiology that occur during clinical CPB. Although it might have been possible to decrease many of these differences by altering the experimental design (e.g., giving insulin to decrease glucose, giving erythrocytes to increase hemoglobin concentration), such interventions would create atypical CPB conditions. Any or all of the differences between CPB and non-CPB animals might contribute toward the adverse effect of CPB on SSEP recovery.

For example, in experiment B, P₉O₂ was greater in CPB animals than in non-CPB animals at later points in the experiment. In theory, greater P₉O₂ could have either beneficial or detrimental effects. Increasing P₉O₂ decreases the time required for CAAE absorption. Using a rabbit CAAE model that was nearly identical to that used in this experiment, Helpes et al. showed that CAAE temporarily occlude cerebral arterioles that measure 50-200 μm in diameter. Cerebral blood flow (CBF) was interrupted for 1-6 min before CAAE were absorbed, passed downstream, or both. Based on a mathematical model of bubble absorption, increasing P₉O₂ from 200 to 300 mmHg would decrease absorption times of 50-200 μm CAAE by, at most, 1-2 min.

Thus greater P₉O₂ in CPB animals would seem unlikely to decrease meaningfully the initial period of vessel occlusion. On the other hand, it is possible that greater P₉O₂ could exacerbate subsequent reperfusion injuries by increasing free radical production.
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Table 3. Systemic Variables: Experiment C (Air Embolism in Heparinized Non-CPB)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time after Air Embolism</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (baseline)</td>
</tr>
<tr>
<td>Epidural temperature (°C)</td>
<td>38.4 ± 0.3</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>75 ± 6</td>
</tr>
<tr>
<td>Central venous pressure (mmHg)</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>pHb</td>
<td>7.41 ± 0.03</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>189 ± 25</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.5 ± 1.2</td>
</tr>
<tr>
<td>Na⁺ (mm)</td>
<td>142 ± 6</td>
</tr>
<tr>
<td>K⁺ (mm)</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>124 ± 58</td>
</tr>
<tr>
<td>Free methohexitol (µg/ml)</td>
<td>0.8 ± 0.4</td>
</tr>
</tbody>
</table>

Values are mean ± SD; n = 6.

In experiment B, CPB animals had greater glucose concentrations than non-CPB animals. Hyperglycemia occurs commonly during CPB, the result of increased levels of "stress" hormones (glucocorticoids, catecholamines, glucagon, growth hormone). Animal studies indicate hyperglycemia exacerbates neurologic injury after temporary cerebral ischemia. Because, in this

Fig. 1. Experiment A. Somatosensory-evoked potential (SSEP) amplitudes over time after 50 µl/kg saline was injected into the internal carotid artery of rabbits undergoing cardiopulmonary bypass (CPB; group A1) and those with normal circulation (non-CPB; group A2).

Fig. 2. Experiment B. Somatosensory-evoked potential (SSEP) amplitudes over time after 50 µl/kg air was injected into the internal carotid artery of rabbits undergoing cardiopulmonary bypass (CPB; group B1), those with normal circulation (non-CPB; group B2). Eleven animals were included in each group.

Fig. 3. Experiment C. Somatosensory-evoked potential (SSEP) amplitudes over time after 50 µl/kg of air was injected into the internal carotid artery of heparinized rabbits that did not have cardiopulmonary bypass (group C). Six animals were included.

hyperoxic increases in free radical production have not been uniformly demonstrated, and studies are inconsistent as to whether postischemic hyperoxia results in worse neurologic outcome. On balance, the small differences in PaO₂ between CPB and non-CPB animals would seem unlikely to have affected outcome.

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model, CAAE are largely cleared from the cerebral circulation within 1–6 min,18,25 it is reasonable to postulate that CAAE behave, at least in part, like temporary focal lesions. Accordingly, hyperglycemia may exacerbate neurologic injury that follows CAAE and may have contributed to the lesser SSEP recovery in CPB animals.

Animals that had CPB were markedly anemic compared with those that did not. In animal models of focal cerebral ischemia, moderate hemodilution (hemoglobin ~10 g/dl) reduces neurologic injury.38–40 In contrast, Reasoner et al.15 and Lee et al.51 found that marked hemodilution (hemoglobin level <10 g/dl) exacerbates neurologic injury in the setting of permanent focal ischemia. In the experiment by Reasoner et al.,15 the adverse effect of marked hemodilution was evident 4 h after onset of ischemia. Therefore the lesser SSEP recovery in CPB animals may have been due to their lesser hemoglobin concentration.

When introduced as a macroscopic bolus, air rapidly breaks up into smaller bubbles.42 This process is called fractionation. As discussed before, injection of air into the rabbit internal carotid artery results in CAAE that occlude pial arterioles (50–200 μm diameter) for 1–6 min. With bubble clearance, microvascular flow is restored, but this is followed by progressive SSEP and CBF abnormalities.18,24,25 In all animals in all experiments, injection of 50 μl/kg air into the internal carotid artery resulted in immediate total loss of the SSEP signal. Thus, in all animals, CBF must have been initially reduced so low as to eliminate SSEP (i.e., CBF ≤20% of baseline43). Without subsequent bubble absorption, clearance, or both, SSEP recovery would not have been possible. A major factor determining bubble fractionation is the magnitude of instantaneous shear force exerted on the bubble surface.44 Because of nonpulsatile flow, CPB would be expected to exert less shear force on the initial air bolus than would occur under pulsatile (normal circulatory) conditions. Therefore, to the extent that nonpulsatile CPB decreases CAAE fractionation, clearance, or both, restoration of CBF and SSEP could be delayed or diminished.

Animals that had CPB had greater baseline free methohexital concentrations than did those that did not. This occurred despite a decreased methohexital maintenance rate in CPB animals. We postulate that hemodilution with initiation of CPB decreased methohexital protein binding,22,45 leading to a greater free fraction of the original loading dose. Ninety minutes after air embolism, free methohexital concentrations were equivalent among groups. Recently, Warner et al.,46 using a rat model of temporary focal ischemia, observed that the protective effect of a barbiturate was not highly dose dependent. A lesser dose of barbiturate, which was associated with less electroencephalographic and metabolic suppression, was equally protective as a larger dose, which resulted in nearly maximal electroencephalographic and metabolic suppression. Given this observation, and the equivalence of free methohexital concentrations at 90 min among groups, the lesser SSEP recovery in CPB animals would seem unlikely to be due to the initially greater free methohexital concentration in this group.

The Potential Effect of Heparin on Cerebral Air Embolism

In experiment B, hyperoxia, hyperglycemia, anemia, or nonpulsatile flow in CPB animals may have contributed to their lesser SSEP recovery compared with the non-CPB group. On the other hand, CPB animals were heparinized, whereas non-CPB animals were not. In a previous non-CPB experiment, we observed that a 2 h heparin infusion at the time of CAAE reduced neurologic impairment 24 h later.11 Therefore, we wondered whether the presence of heparin in CPB animals might improve their outcome compared with that in nonheparinized non-CPB animals. To learn whether heparin might affect SSEP recovery after CAAE, we performed an additional experiment in non-CPB animals that received heparin in the same dose as CPB animals (group C). We observed in heparinized non-CPB animals that, despite initial loss of signal, SSEP recovery was present 5 min after CAAE. Although highly variable in magnitude, this rapid SSEP recovery was in stark contrast to nonheparinized non-CPB animals, in which there was no SSEP recovery at 5 min.

Utrastructural17,52 and functional studies51,52 indicate that CAAE damage cerebral arteriolar endothelium. As a result, fibrin,47,48 platelets,47,53,54 and neutrophils17,47,55 accumulate at air-damaged endothelium. In dogs, radio-labeled neutrophils focally accumulate in the brain within 1 h after CAAE.55 Neutrophil accumulations correspond to areas of low CBF.55 Platelet inhibitors (indomethacin, prostaglandin I2) do not affect CBF and SSEP abnormalities that follow CAAE, nor do they prevent neutrophil accumulation.56 In contrast, studies in dogs17 and rabbits18 show that neutrophil depletion before CAAE prevents abnormalities in SSEP and CBF that ordinarily occur over the first 1–2 h after CAAE. Using our rabbit model of CAAE, we recently showed that doxycycline, an antibiotic that inhibits neutrophil adhe-
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sion and enzymatic activities, improves SSEP and neurologic recovery. The benefits of doxycycline could be detected within 1 h of CAAE. Thus CBF, SSEP, and neurologic abnormalities that follow CAAE appear to be largely neutrophil mediated and to be initiated within the first hour after CAAE. In vitro, inhibition of selectin-mediated neutrophil adhesion has been shown to occur at heparin concentrations achieved in our experiments. In vitro, intravenous heparin inhibits leukocyte rolling in rabbit mesenteric venules and inhibits neutrophil migration into rabbit skin in response to a variety of inflammatory and chemotactic stimuli. Thus, heparin may protect the brain against CAAE by inhibiting neutrophil adhesion to air-injured cerebral endothelium.

Nevertheless, despite equivalent doses of heparin, CPB animals did not exhibit the early SSEP recovery present in heparinized non-CPB animals. Therefore, it appears that, at least in part, CPB may negate the beneficial effects of heparin against CAAE. As discussed previously, hyperoxia, hyperglycemia, anemia, and nonpulsatile flow may have contributed to the adverse effect of CPB. However, an additional factor to consider is the inflammatory response of CPB. Cardiopulmonary bypass results in nearly immediate systemic complement activation. Notably, this occurs despite the presence of heparin. Activated complement fragments may promote neutrophil adherence to air-damaged cerebral endothelium via multiple mechanisms. For example, C5a increases neutrophil expression of its major endothelial adhesion receptor CD11b/CD18. C5a also increases endothelial P-selectin expression. Activated complement inhibits acetylcholine-mediated vessel relaxation. Because acetylcholine vasodilation is mediated via nitric oxide, the cerebral vasculature may have decreased endothelial nitric oxide synthesis during CPB. Decreased endothelial nitric oxide increases leukocyte-endothelial interactions. Finally, C5a also decreases leukocyte deformability. Reduced deformability would promote neutrophil entrapment and adhesion at sites of injured endothelium. Collectively, increased neutrophil or endothelial adhesivity during CPB, which occurs despite the presence of heparin, may exacerbate the inflammatory mechanisms of CAAE.

Conclusions

We found that SSEP recovery after CAAE is no better (and is probably worse) during CPB than during normal (nonheparinized non-CPB) circulation. Several characteristics of CPB may have contributed to the apparently greater adverse effect of CAAE on the brain, including hyperoxia, hyperglycemia, anemia, nonpulsatile flow, and complement activation. The adverse effect of CPB occurs despite the presence of heparin, which improved neurologic outcome in a previous non-CPB study in this model and also appeared to improve SSEP recovery after CAAE in non-CPB animals. Thus CAAE are just as injurious to the brain (if not more so) during CPB as they are with normal circulation. This conclusion is consistent with the observation that CAAE occurring during clinical CPB result in a dose-dependent deterioration of postoperative neurologic status. Thus, therapies in addition to heparin are needed during CPB to reduce neurologic injury from CAAE.

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