Blood–Brain Barrier Disruption After Cardiopulmonary Resuscitation in Immature Swine

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We investigated blood–brain barrier permeability in 2–3-week-old anesthetized pigs during and after cardiopulmonary resuscitation. We assessed permeability by tissue uptake of radiolabeled aminoisobutyric acid, after correcting for plasma counts in tissue with radiolabeled inulin. Among 14 regions examined, the transfer coefficient of aminoisobutyric acid in nonischemic control animals ranged from 0.0018 ± 0.0001 ml/g/min in diencephalon to 0.0049 ± 0.0003 ml/g/min in cervical spinal cord. After 8 minutes of cardiac arrest followed by either 10 or 40 minutes of continuous sternal compression, there was no increase in the transfer coefficient. Likewise, during the immediate period after ventricular defibrillation, there was no increase in transfer coefficient despite the brief, transient hypertension. However, after 8 minutes of arrest, 6 minutes of cardiopulmonary resuscitation, and 4 hours of spontaneous circulation, the transfer coefficient was significantly increased by 59–107% in 10 of 11 regions rostral to the pons. Plasma volume in tissue measured by inulin was not elevated, suggesting that the increased transfer coefficient was not due to increased surface area. Thus, after an 8-minute period of complete ischemia, the blood–brain barrier remains intact during and immediately after resuscitation despite large vascular pressure fluctuations. However, in contrast to previous work on adult dogs, immature pigs are prone to a delayed increase in permeability, thereby allowing circulating substances greater access to the brain. (Stroke 1991;22:477–483)

Blood–brain barrier function after cardiac arrest is important in limiting access to the brain of endogenously released humoral substances and exogenously administered drugs that could affect cerebral metabolism or vascular smooth muscle. We have reported previously that external cardiopulmonary resuscitation (CPR) does not result in disruption of the blood–brain barrier as assessed by the transfer coefficient of the small molecule α-aminoisobutyric acid (AIB).1 This finding was unexpected because cyclic chest compression produces large pulse pressures in the range of 60–100 mm Hg in the aorta and 30–40 mm Hg in the cerebral venous sinuses.2,3 Large pulse pressures in a postischemic, vasodilated bed could mechanically disrupt the blood–brain barrier, but the concurrent, phasic increase in cerebrospinal fluid pressure apparently limits the increase in cerebral venous transmural pressure.

These previous studies were performed on mature dogs. We also have reported previously in a pediatric model of CPR using immature pigs that near-normal levels of cerebral blood flow can be generated with external chest compression when combined with continuous infusion of high doses of epinephrine.4 However, as in adult dogs, large phasic increases in aortic and sagittal sinus pressure occurred.4,5 Although endothelial tight junctions are present at birth in most species,6 they might not be as structurally resilient to withstand large pulse pressures as in mature animals. Furthermore, large cerebral artery resistance vessels that ordinarily minimize changes in microcirculatory pressures in adult animals7 may not be as prominent in younger animals, which normally have a lower aortic pressure and cerebrovascular resistance.8

In the present study, we determined whether the blood–brain barrier transfer coefficient of AIB is as
well maintained in immature piglets as it is in adult dogs during CPR after an 8-minute period of cardiac arrest. We also assessed blood–brain barrier integrity at 3 minutes and at 4 hours after return of spontaneous circulation. Increased blood–brain barrier permeability has been described in adult animals after prolonged ischemia,9–11 but postischemic changes in immature animals have not been well documented.

**Materials and Methods**

Twenty-five piglets 2–3 weeks old (4.5–5.5 kg) were anesthetized with pentobarbital (35–40 mg/kg i.p.) and ventilated with a volume-cycled ventilator through an endotracheal tube secured by a tracheostomy. Fractional inspired oxygen was 0.3–0.5. End-tidal CO₂ was monitored to maintain PaCO₂ at 35–40 mm Hg. If the animals began to move, additional pentobarbital was given intravenously. Generally, 7–20 mg/kg i.v. was required to maintain a depth of anesthesia adequate for surgery. Saline-filled catheters were advanced through a femoral artery into the thoracic aorta and through a femoral vein into the right atrium. Catheters also were advanced through the axillary artery and vein into the subclavian artery and vein, respectively. At the end of surgery, the animals were paralyzed with pancuronium (0.1 mg/kg), and heparin (1,000 units) was administered.

Ventricular fibrillation was induced by passing a 60-Hz current through a 4F pacing wire that was advanced into the right heart via a femoral vein. Ventilation was stopped. After 8 minutes of arrest, external chest compression was performed over the sternum with a pneumatic chest compressor (Thumper, Michigan Instruments, Grand Rapids, Mich.). The chest compressor and a pressure-limited ventilator were synchronized by a microprocessor. Chest compressions were performed at a rate of 100 per minute, with a compression duration of 40% of the total cycle time. Compression force was set at 40–50 N to produce a cyclic sternum displacement equivalent to approximately 20% of the anteroposterior diameter. Ventilation was provided with 100% oxygen after every fifth chest compression at an airway pressure of 30–35 cm H₂O.

We studied five groups of five animals each in which AIB was allowed to circulate for 10 minutes before stopping brain perfusion. In a control group, group 1, measurements were made during spontaneous circulation without prior cardiac arrest. In group 2, ventricular fibrillation, documented by the aortic pressure tracing, lasted 8 minutes before CPR began. Then, CPR was continued for 10 minutes without attempting ventricular defibrillation. To assess the effect of prolonged CPR (group 3), we followed 8 minutes of ventricular fibrillation with 40 minutes of CPR, again without attempts to defibrillate. To assess the effect of the immediate return of spontaneous circulation after cerebral ischemia and CPR (group 4), we followed 8 minutes of ventricular fibrillation with 6 minutes of continuous CPR. Ventricular defibrillation then was achieved by external countershock within four attempts, and AIB was injected 3 minutes later. To assess any delayed, postischemic effect (group 5), we again followed 8 minutes of ventricular fibrillation, followed by 6 minutes of CPR, with up to four defibrillatory attempts. After 4 hours of reperfusion, AIB was injected.

Epinephrine was given as a bolus of 10 µg/kg into the right atrium at the onset of CPR in all animals in groups 2–5. An infusion of 4 µg/kg/min diluted in saline was administered into the subclavian vein at a volumetric rate of 1.9 ml/min for the duration of CPR. This dose and volume maintains cerebral perfusion pressure at levels sufficient for near-normal cerebral blood flow for 20 minutes of CPR. After defibrillation, in groups 4 and 5, the infusion of epinephrine was decreased in approximately half logarithmic increments at 1-minute intervals when the mean aortic pressure was greater than 70 mm Hg. With this criterion, the infusion was stopped by 30 minutes after resuscitation in all animals.

We recorded pressure from the intrathoracic aorta with the transducer referenced to the level of the right atrium. We measured arterial blood gases and pH with Radiometer ABL 3 electrodes and analyzer (Copenhagen).

We assessed permeability of the blood–brain barrier function using a low molecular weight tracer, ³⁴C-AIB (molecular weight, 104) (Dupont–New England Nuclear Products, Boston). Animals received an AIB injection of 150 µCi i.v. (specific activity, 40–60 mCi/mmol) into the right atrium to determine its transfer coefficient (Kₐ) into the brain. In group 2, AIB was given 3 minutes after arrest. In group 3, AIB was given 30 minutes after the onset of CPR. In group 4, AIB was given 3 minutes after defibrillation. In group 5, AIB was given 4 hours after defibrillation. To correct for AIB in the plasma space, we administered ³⁴H-inulin (Dupont–New England Nuclear) as a bolus of 150 µCi i.v. (150 µl) into the right atrium in every animal exactly 8 minutes after the injection of AIB. Timed arterial blood samples (1 ml) were drawn from the axillary artery catheter at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 9, and 10 minutes after the injection of AIB. The animals were killed immediately after the 10-minute blood sample by either stopping CPR (groups 2 and 3) or by injection of potassium chloride (groups 1, 4, and 5).

At the end of each experiment, the blood samples were centrifuged and 50 µl plasma was pipetted into glass counting vials. An autopsy was performed, and the brain was removed for dissection. Eight cortical samples weighing 200 mg each were obtained from each of the primary supply regions of the anterior, middle, and posterior cerebral arteries, and from the anterior-middle and middle-posterior watershed regions. Four samples were dissected from the cerebel- lum. Two samples were dissected from the cervical spinal cord, medulla, pons, midbrain, diencephalon, hippocampus, and caudate nucleus. One sample was
dissected from the superior colliculus and pituitary. All tissue specimens were placed in glass vials with 2 ml Protosol (Dupont-New England Nuclear) to dissolve the tissue. The tissue vials were placed in a water bath at 48–50°C overnight. Ten milliliters of Biocount (Research Products International Corp., Mt. Prospect, Ill.) was used for this double-label scintillation cocktail. Glacial acetic acid (0.05 ml) was added to neutralize the solution and minimize chemiluminescence. Samples were kept at room temperature overnight before beta counting was performed with a liquid scintillation spectrometer (model LS 3801, Beckman Instruments, Schiller Park, Ill.). Disintegrations per minute (dpm) were obtained using a double isotope technique corrected for quench and background by the external standard method. The transfer coefficient for AIB was calculated based on the equations of Ohno et al\textsuperscript{12} and Blasberg et al\textsuperscript{13}:

\[
K_i = \frac{14C(T) - [3H(T) \times 14C(P)\bar{H}(P)]}{10 \int 14C(P)dt}
\]

where \(K_i\) is the transfer coefficient (ml/g/min), \(14C(T)\) is the concentration of AIB in the brain (dpm/g), \(3H(T)\) is the concentration of inulin in the brain (dpm/g), \(14C(P)\) is the concentration of AIB in the 10-minute plasma sample (dpm/ml), \(3H(P)\) is the concentration of inulin in the 10-minute plasma sample (dpm/ml), and \(\int_0^{10} 14C(P)dt\) is the integrated arterial plasma concentration for AIB over the 10-minute sampling time (min \times dpm/ml).

Differences in \(K_i\) values among groups were evaluated by one-way analysis of variance. Comparisons to the control group (group 1) were made with the Dunnett’s test at the 0.05 significance level. Values are expressed as mean±SEM.

**Results**

Figure 1 shows data for 14 brain regions, with the bar representing the mean \(K_i\) for each group and the closed circles representing the individual data (\(n=5\) per group). In the control group, \(K_i\) values ranged from 0.002 to 0.005 among regions. In groups 2, 3, and 4, in which AIB was injected at the start of CPR, at 30 minutes of CPR, and at 3 minutes after defibrillation, respectively, \(K_i\) values were not signif-
FIGURE 2. Mean±SEM of $^3$H-inulin tissue-to-plasma ratio for cervical spinal cord (SC), cerebellum (CER), medulla (MED), pons (PONS), midbrain (MID), superior colliculus (COLL), diencephalon (DIE), hippocampus (HIP), caudate nucleus (CAU), anterior cerebral (AC), middle cerebral (MC), and posterior cerebral (PC) artery regions, and anterior-middle (AMW) and posterior-middle (PMW) artery watershed regions. 1, control group; 2, 8 minutes ischemia and 10 minutes cardiopulmonary resuscitation; 3, 8 minutes ischemia and 40 minutes cardiopulmonary resuscitation; 4, 3 minutes after resuscitation; 5, 4 hours after resuscitation. There were no significant differences among groups by one-way analysis of variance for any region.

Significantly different from those in the control group. However, in group 5, when AIB was injected 4 hours after defibrillation, $K_v$ was significantly elevated in most regions. As a percentage of the mean group 1 value, $K_v$ was significantly increased to 191% in pons; 191% in midbrain; 175% in superior colliculus; 207% in diencephalon; 159% in hippocampus; 210% in caudate nucleus; 160% in the anterior-middle watershed region; and 171%, 162%, and 163% in the anterior, middle, and posterior cerebral arterial territories, respectively.

The $K_v$ values for the pituitary gland ranged from 0.030±0.009 to 0.186±0.035 in the five groups. These values were 10–40 times higher than corresponding values for other brain regions. This indicates that AIB can be sequestered in a piglet brain region devoid of a blood–brain barrier and also indicates that the label was delivered intracranially in each animal.

Changes in surface area may obscure changes in AIB permeability. Surface area is proportional to the number of perfused microvessels and the square of their diameter. Hence, surface area may be expected to vary as a nonlinear function of tissue plasma volume. We estimated brain tissue plasma volume using the ratio of tissue $^3$H-inulin to plasma $^3$H-inulin obtained just before stopping brain perfusion. This provides an estimated tissue plasma volume because the molecular weight of inulin is about 70 times that of AIB and because its circulation time was only 2 minutes. Figure 2 shows mean values for the five groups. There were no significant differences in the inulin ratio among groups for any brain region. In group 5 in particular, there was no change in the estimated plasma volume that could account for the increase in $K_v$ of AIB.

Mean aortic blood pressure values at the midpoint of AIB circulation as well as at earlier times in the protocol for the five groups are shown in Table 1. Mean aortic pressure was below prearrest values during CPR and elevated above prearrest values during the first few minutes after defibrillation. The maximum aortic systolic pressure after resuscitation in groups 4 and 5 was 186±4 and 190±17 mm Hg, respectively. These maximum pressures occurred at 2.6±1.0 and 1.8±0.6 minutes after resuscitation in the two respective groups.
TABLE 1. Mean Aortic Pressure and Arterial Blood Gases

<table>
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<tr>
<th>Group</th>
<th>MAP</th>
<th>pH</th>
<th>Pco₂</th>
<th>Pa₀₂</th>
<th>MAP</th>
<th>pH</th>
<th>Pco₂</th>
<th>Pa₀₂</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>81±5</td>
<td>7.43±0.02</td>
<td>37±2</td>
<td>194±24</td>
<td>67±12</td>
<td>7.16±0.06</td>
<td>35±8</td>
<td>106±40</td>
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<td>2</td>
<td>85±9</td>
<td>7.40±0.04</td>
<td>35±2</td>
<td>177±14</td>
<td>57±4</td>
<td>7.23±0.05</td>
<td>48±9</td>
<td>112±42</td>
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<tr>
<td>3</td>
<td>113±18</td>
<td>7.45±0.03</td>
<td>35±3</td>
<td>160±18</td>
<td>57±8</td>
<td>7.23±0.05</td>
<td>48±9</td>
<td>112±42</td>
</tr>
<tr>
<td>4</td>
<td>103±13</td>
<td>7.39±0.02</td>
<td>41±2</td>
<td>179±11</td>
<td>55±5</td>
<td>7.39±0.02</td>
<td>41±3</td>
<td>146±8</td>
</tr>
<tr>
<td>5</td>
<td>87±6</td>
<td>7.44±0.03</td>
<td>33±3</td>
<td>146±5</td>
<td>48±2</td>
<td>7.30±0.07</td>
<td>33±6</td>
<td>162±64</td>
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<td>After resuscitation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Group 1</td>
<td>136±5</td>
<td>7.12±0.04</td>
<td>41±3</td>
<td>146±8</td>
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<tr>
<td>Group 2</td>
<td>116±18</td>
<td>7.19±0.04</td>
<td>39±4</td>
<td>101±7</td>
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<tr>
<td>Group 3</td>
<td>91±10</td>
<td>7.31±0.03</td>
<td>35±2</td>
<td>137±33</td>
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<td>Group 4</td>
<td>89±18</td>
<td>7.34±0.05</td>
<td>42±4</td>
<td>96±18</td>
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</table>

Values are mean±SEM. Group 1, control; group 2, 8 minutes ischemia and 10 minutes cardiopulmonary resuscitation (CPR); group 3, 8 minutes ischemia and 40 minutes CPR; group 4, 3 minutes after resuscitation; group 5, 4 hours after resuscitation. MAP, mean aortic pressure.

None of the animals was hypoxic prearrest, during CPR, or after resuscitation in any of the experimental protocols. In addition, there were no differences among groups in arterial PCO₂ level at the time of injection (Table 1).

Discussion

There are three major findings of this study on immature swine after a period of 8 minutes of complete ischemia produced by cardiac arrest. First, the mechanical effects of external chest compression for as long as 40 minutes do not cause major blood-brain barrier disruption. Second, the brief hypertension immediately after defibrillation does not lead to immediate blood-brain barrier disruption. Third, there is delayed blood-brain barrier dysfunction 4 hours after reperfusion.

We anticipated blood-brain barrier disruption during CPR because of the mechanical effects of vascular pressure fluctuations in an ischemic brain. With each chest compression, there are large phasic increases in cerebral venous pressure of as much as 30-40 mm Hg. Ordinarily with spontaneous circulation, blood-brain barrier leakage at pial venular sites is associated with increases in venular transmural pressure of 20 mm Hg or less. However, we did not observe a significant increase in AIB permeability during CPR in this study. We attribute this result to the simultaneous increase in cerebrospinal fluid pressure during the compression phase of CPR. The increase in cerebrospinal fluid pressure is partly due to transmission of intrathoracic pressure to the cerebrospinal fluid via vertebral foramina. With simultaneous and equivalent increases in cerebrospinal fluid pressure and cerebral venous pressure, there would be no dynamic change in the transmural pressure arising from venous hypertension. When cerebrospinal fluid was drained in nonischemic dogs in our previous study, an increase in sagittal sinus venous pressure resulted in an increase in Kᵩ of AIB. It also is possible that longer durations of cardiac arrest are required before any mechanical effects on blood-brain barrier function are evident during CPR. We selected an 8-minute period of arrest because longer periods were associated with a greater failure rate in resuscitating the heart. Finally, prolonged or intermittent asphyxia, which is a more common cause of cardiac arrest in infants, may be more apt to cause blood-brain barrier disruption than 8 minutes of ventricular fibrillation.

The period immediately after resuscitation is one in which the blood-brain barrier is prone to disrup-
tion because of postischemic cerebral vasodilation in the presence of arterial hypertension. In adult dogs, Arai et al. showed extravasation of Evans' blue dye associated with hypertension after resuscitation. Differences between this study and ours may be due to the severity and duration of hypertension, differences in resuscitation techniques, or diffusion of Evans' blue during 1-month formalin fixation. In our study, the duration of hypertension was generally brief, lasting less than 1 minute, and often occurred before the injection of AIB at 3 minutes after resuscitation. By 5 minutes after resuscitation, mean arterial pressure decreased to 136±5 mm Hg (Table 1). Furthermore, the duration of postischemic vasodilation may be brief in the piglet and thereby minimize the duration of increased microvascular pressure. Last, it is also possible that high levels of circulating epinephrine protect the endothelium from oxygen radical damage because epinephrine is oxidized by superoxide anion.

In our previous study of blood–brain barrier permeability in adult dogs, we did not show disruption 4 hours following CPR after 8 minutes of ischemia. In the present study on piglets with an identical time course protocol, we demonstrated consistent blood–brain barrier disruption 4 hours after defibrillation. There may be an age-related difference in the timing of when a delayed increase in blood–brain barrier permeability may occur during reperfusion, and this timing probably depends on ischemic duration. We did not study the effect on blood–brain barrier permeability of ischemia longer than 4 hours after resuscitation, and we cannot exclude a delayed increase in permeability at a later time. In models of global and focal ischemia, delayed increases in blood–brain barrier permeability during reperfusion are generally associated with long ischemic durations (30–60 minutes). Therefore, the observed increase in Kj in piglets after only 8 minutes of complete ischemia may be more prone to delayed vasogenic edema. Although this delayed increase in Kj is most likely attributable to the period of complete ischemia, we cannot exclude delayed effects due to the mechanics of CPR or the brief period of hypertension after cardiac resuscitation. The precise mechanism for the increase in Kj is unknown.

The increase in Kj was detected in most of the cerebrum, diencephalon, hippocampus, caudate nucleus, midbrain, and pons. Even in those regions in which the increase in Kj was not significant, such as the cerebellum, medulla, and the posterior-middle watershed area, three of the five animals had higher Kj values than any of the control animals. Thus, the increase in blood–brain barrier permeability did not exhibit a great deal of regional specificity.

Various investigators studying a variety of species have shown that the blood–brain barrier is structurally intact at birth. In the rat, which has a relatively short gestation, endothelial tight junctions are present at birth, although there are some differences between the newborn and adult rat in specific transport systems. In the pig, myelination appears to occur primarily during the first three postnatal weeks, and one would anticipate that the blood–brain barrier is structurally intact by this time. Our data showing that the Kj for AIB in the cerebrum in the control group was approximately 10 times less than that of the pituitary supports this assumption. Moreover, the prearrest values for Kj of 0.003 ml/g/min in most brain regions is similar to those reported in mature gerbil (0.002–0.005), rat (0.002–0.004), and dog (0.002–0.007).

In conclusion, after 8 minutes of complete ischemia and 6 minutes of CPR in immature pigs, we found evidence of a delayed increase in blood–brain barrier permeability to a small molecule 4 hours after resuscitation. However, during either 10 or 40 minutes of continuous CPR or immediately after defibrillation, there was no evidence for disruption of the barrier. Therefore, adrenergic agonists, which are frequently used during CPR or after cardiac arrest, are not likely to cross the blood–brain barrier at these times. If asphyxial arrest is similar to fibrillatory arrest, then blood pressure management with vasopressor agents several hours after pediatric resuscitation may lead to their access to the brain, where they could adversely affect cerebral metabolism or cerebrovascular smooth muscle tone. Thus, blood–brain barrier disruption after CPR might potentially lead to undesirable effects by circulating exogenous or endogenous agents.

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References

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