Blood–Brain Barrier Integrity During Cardiopulmonary Resuscitation in Dogs

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Blood–brain barrier integrity during cardiopulmonary resuscitation may be important because of the potential effects of adrenergic agonists administered during arrest on cerebral metabolism and the cerebral vasculature. As an index of blood–brain barrier permeability to small molecules, we measured the brain uptake of \[^{14}C\]α-aminoisobutyric acid during a 10-minute period in 25 anesthetized dogs. To correct for the amount of carbon-14 label in the plasma space, we administered \[^{3}H\]inulin 2 minutes before death. The mean transfer coefficient in 14 brain regions of five control dogs ranged from 0.002 to 0.007 ml/g/min. After 8 (n = 15) or 15 (n = 5) minutes of cardiac arrest, external chest compression was instituted to maintain aortic blood pressure above 60 mm Hg. The transfer coefficient was not elevated during chest compression (n = 10), immediately following defibrillation (n = 5), or 4 hours after resuscitation (n = 5); in some brain regions the transfer coefficient decreased. However, the decrease in the transfer coefficient was proportional to the decrease in the cerebral plasma volume as measured by the ratio of the \[^{3}H\]inulin concentration in the tissue to that in the plasma. Thus, it is unlikely that a decrease in capillary surface area masked an increase in blood–brain barrier permeability. Therefore, we found no evidence of blood–brain barrier disruption during or after cardiopulmonary resuscitation in dogs. Despite the large phasic increases in sagittal sinus pressure associated with external chest compression, concurrent increases in cerebrospinal fluid pressure apparently protect the microcirculation from increased transmural pressure.

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Integrity of the blood–brain barrier (BBB) during cardiopulmonary resuscitation (CPR) is important due to the potential effects on cerebral metabolism and vascular smooth muscle evoked by a host of humoral substances released after cardiac arrest and by administered drugs such as adrenergic pressor agents. Blood–brain barrier dysfunction could arise from either the mechanical vascular effects associated with CPR or from the biochemical effects associated with complete cerebral ischemia during cardiac arrest.

During chest compression there are large phasic fluctuations in both arterial and cerebral venous pressures\(^1\) that could mechanically disrupt the BBB, particularly in the maximally vasodilated bed. Immediately following CPR, rapid restoration of blood pressure in a vasodilated bed may also increase BBB permeability, as observed in some models of focal ischemia.\(^2\) In addition, following prolonged global ischemia BBB permeability may increase several hours into the reperfusion period.\(^3\) Thus, there are several critical periods associated with CPR during which increased BBB permeability might be anticipated: during chest compression, during the hyperemic phase immediately after defibrillation, and during the delayed hypoperfusion phase.

In one study, extravasation of Evans blue was observed during the transient hypertensive period following defibrillation.\(^4\) However, BBB integrity during a stable period of continuous external chest compression after cardiac arrest has not been well quantified. We used the \[^{14}C\]α-aminoisobutyric acid (AIB) technique\(^5\) in dogs to assess BBB integrity during a hemodynamically stable and reproducible period of external chest compression. We also examined BBB integrity during the hyperemic phase immediately following defibrillation and during the delayed hypoperfusion period 4 hours after CPR.

Materials and Methods

Twenty-five mongrel dogs weighing 18–23 kg were anesthetized with 50 μg/kg i.v. fentanyl and 10 mg/kg...
i.v. pentobarbital and then ventilated through an endotracheal tube secured by a tracheostomy. Fractional inspired O₂ was 30–50%. End-tidal CO₂ was monitored to maintain PacO₂ at 35–40 mm Hg. If the dog began to move, additional pentobarbital was given; generally 5–10 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. A 4-French pacing wire was passed into the right heart for induction of ventricular fibrillation. Catheters were also passed through the axillary artery and vein into the subclavian artery and vein for sampling of blood and infusion of epinephrine, respectively. At the end of surgery the dog was paralyzed with 0.1 mg/kg pancuronium, and 4,000 units heparin was administered.

Ventricular fibrillation was induced by passing a 60-Hz current through the pacing wire in the right heart. External chest compression to maintain aortic blood pressure above 60 mm Hg was performed over the sternum with a pneumatic chest compressor (Thumper, Michigan Instruments, Grand Rapids, Mich.). Chest compressions were performed at a rate of 40/min, with compressions lasting 50% of the total cycle time. Compression force was set at 110–120 N to produce a cyclic sternal displacement equivalent to 15–20% of the anteroposterior diameter. Simultaneous ventilation at high airway pressure (75–85 mm Hg) was provided by a pressure-limited ventilator during the first 40% of each cycle. The dogs were ventilated with a mixture of 95% O₂ and 5% CO₂ during CPR to prevent hypoxia and avoid hypocapnia.

We studied five groups. In group 1 (n=5), the control group, measurements were made during spontaneous circulation without prior cardiac arrest. In group 2 (n=5), cardiac fibrillation, documented by the aortic blood pressure tracing, lasted 8 minutes before CPR commenced; CPR was then continued for 10 minutes without attempting ventricular defibrillation. In group 3 (n=5), 8 minutes of cardiac fibrillation was followed by 6 minutes of CPR. Ventricular defibrillation was achieved within four attempts by external counter-shock, and the dogs were killed 10 minutes after defibrillation by the intravenous injection of KCl. In group 4 (n=5), 8 minutes of cardiac fibrillation was followed by 6 minutes of CPR and then by defibrillation; the dogs were killed 4 hours later by the intravenous injection of KCl. In group 5 (n=5), cardiac fibrillation lasted 15 minutes before CPR commenced; CPR was performed for 10 minutes without attempts at defibrillation.

Epinephrine was given as a 10 μg/kg bolus into the right atrium at the onset of CPR in groups 2–5. For the duration of CPR an infusion of 4 μg/kg/min i.v. epinephrine in saline was administered at a volumetric rate of 3.9 ml/min. This dose maintains cerebral perfusion pressure at levels sufficient for near-normal cerebral blood flow. Following defibrillation, the epinephrine infusion was decreased in half-logarithmic steps at 1-minute intervals when the mean aortic blood pressure was >90 mm Hg. Infusion was stopped by 12 minutes after defibrillation in all dogs.

To test the sensitivity of the AIB technique to elevations in cerebral venous pressure, we used the venous outflow technique of Rapela and Green⁶ to isolate the cerebral venous drainage in two additional dogs. A screw clamp on the venous outflow side of the circuit was used to raise the cerebral venous pressure to 30 mm Hg in one dog and 75 mm Hg in the other. In addition, a 16-gauge catheter was inserted into the cisterna magna to drain cerebrospinal fluid. After 3–4 minutes of stable pressures, AIB was injected. The dogs were killed 10 minutes later.

Pressures were recorded from the intrathoracic aorta and right atrium with Statham 23 Db transducers (Hato Rey, Puerto Rico) referenced to the level of the right atrium. Arterial blood gases and pH were obtained before cardiac arrest in all five groups, during CPR in groups 2–5, and after defibrillation in groups 3 and 4.

Ten minutes before death, 250 μCi of the low-molecular-weight tracer AIB (molecular weight 104, specific activity 40–60 mCi/mmol; Du Pont—New England Nuclear Products, Boston, Mass.) was injected intravenously. In groups 2 and 5 AIB was given at the onset of CPR after vascular pressures stabilized (within 20 seconds), in group 3 it was given immediately after defibrillation, and in group 4 it was given 4 hours after defibrillation. To correct for the amount of AIB in the plasma space, 250 μCi (0.25 ml) [³H]inulin (Du Pont—New England Nuclear Products) was injected intravenously exactly 8 minutes after the injection of AIB. Arterial blood samples were drawn from the axillary artery catheter 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.

Two tissue samples weighing 200 mg apiece were dissected from the hippocampus, caudate nucleus, cervical spinal cord, medulla, pons, midbrain, and diencephalon; one sample each was dissected from the superior colliculus and pituitary gland; and four samples were dissected from the cerebellum. Eight cortical samples were obtained from each of the primary supply regions of the anterior, middle, and posterior cerebral arteries and from the secondary anterior—middle and posterior—middle cerebral arterial watershed regions. All samples were placed in glass vials. Two milliliters of Protosol (Du Pont—New England Nuclear Products) was added to each vial to dissolve the tissue sample, and the vials were placed in a water bath at 48–50⁰ C overnight. Ten milliliters of Biocount (Research Products International Corp., Mt. Prospect, Ill.) was used for this double-label scintillation cocktail on 50-μl aliquots of tissue and plasma. Glacial acetic acid (0.05 ml) was added to neutralize the solution and minimize chemiluminescence. Samples were kept at room temperature overnight before counting β emissions (LS 3801, Beckman Instruments, Inc., Schiller Park, Ill.).
Disintegrations per minute (dpm) of both β-emitting substances were obtained using a double-isotope technique corrected for quench and background by the external standard method. Using the equation of Ohno et al as modified by Blasberg et al, the transfer coefficient ($K_i$ in milliliters per gram per minute) for AIB was calculated as 

\[ \frac{14C(T) - (3H(T) \times 14C(P))}{3H(P)} \times \int_{0}^{10} 14C(P) \, dt, \]

where $14C(T)$ is the concentration of AIB in the tissue sample at the end of the experiment (dpm per gram), $3H(T)$ is the concentration of $[3H]$inulin in the tissue sample at the end of the experiment (dpm per gram), $14C(P)$ is the concentration of AIB in the 10-minute plasma sample (dpm per milliliter), $3H(P)$ is the concentration of $[3H]$inulin in the 10-minute plasma sample (dpm per milliliter), and $\int_{0}^{10} 14C(P) \, dt$ is the integrated arterial plasma concentration of AIB during the 10-minute sampling time (minutes×dpm per milliliter).

We also calculated a corrected $K_i$ ($K_i'$) that normalized for differences in plasma volume among groups as 

\[ (a/b)K_i, \]

where $a$ is the mean ratio of the concentration of $[3H]$inulin in the tissue to that in the plasma for the five dogs in group 1 and $b$ is the $[3H]$inulin tissue-to-plasma ratio in each dog in the experimental group. This normalization assumes a simple linear relation between capillary surface area and plasma volume among dogs, which is a reasonable first approximation if the predominant mechanism of decreased plasma volume is a decrease in perfused capillary density and if the changes in microvascular diameter and hematocrit are small.

For each brain region, $K_i$ is expressed as mean±SEM. Differences among groups were evaluated by one-way analysis of variance. Comparisons with group 1 were made using Dunnett’s test with a probability value of 0.05.

**Results**

The profiles of AIB plasma concentrations for the five groups generally had similar shapes (Figure 1). However, as a result of the low cardiac output during CPR, peak plasma concentrations of AIB were higher and the subsequent declines were slower in groups 2 and 5. These higher plasma concentrations of AIB theoretically have no effect on $K_i$ because they are sufficiently low to remain on the linear portion of the uptake-concentration curve and because the greater amount of residual carbon-14 label in plasma at 10 minutes is corrected for by the $[3H]$inulin tissue-to-plasma ratio. The latter assumes that the uptake of $[3H]$inulin is negligible compared with that of AIB. This assumption appears reasonable because the molecular weight of inulin is 300 times that of AIB and because the exposure to $[3H]$inulin lasted only 2 minutes.

Mean and individual $K_i$ values for 14 brain regions in the five groups are shown in Figure 2. For each brain region the mean $K_i$ values for individual dogs in groups 2–5 was not higher than the mean $K_i$ value in group 1. Only one dog (in group 3) had an individual $K_i$ value higher than the mean $K_i$ value in group 1 for any brain region. This occurred in the spinal cord.
cerebellum, medulla, pons, and midbrain. For some brain regions mean Kᵢ in an experimental group was significantly lower than that in group 1. During CPR in groups 2 and 5, mean Kᵢ was significantly lower for all primary and secondary cerebral artery regions, the hippocampus, midbrain, and diencephalon. In group 5, mean Kᵢ for the cerebellum was also significantly lower. Immediately after defibrillation in group 3, mean Kᵢ was significantly lower for the anterior and posterior cerebral artery regions, the anterior-middle and posterior-middle cerebral artery watershed areas. Four hours after CPR in group 4, mean Kᵢ was significantly lower for all primary and secondary cerebral artery regions, the midbrain, and the superior colliculus.

Mean Kᵢ for the pituitary gland ranged from 0.093±0.017 to 0.036±0.005 in the five groups (data not shown). These values are 10–20 times those for the other brain regions. This result was expected because of the lack of a BBB in the pituitary gland and indicates that the radiolabel was delivered intracranially in each dog.

Kᵢ depends not only on BBB permeability but also on capillary surface area; hence, a decrease in surface area may account for the low postischemic Kᵢ. Capillary surface area is a function of the number of perfused microvessels and the square of their diameter, and thus, surface area may be expected to vary as a nonlinear function of plasma volume. We estimated plasma volume for each brain region using the [³H]inulin tissue-to-plasma ratio. Mean values for 14 brain regions in the five groups are shown in Table 1. For each brain region, this ratio in the experimental groups was less than that in group 1. For example, for the middle cerebral artery region, the [³H]inulin tissue-to-plasma ratio in groups 2–5 ranged from 58% to 75% of that in group 1. Kᵢ for that region in the experimental groups had a similar range of 42–78% of the group 1 value (Figure 2).

Values of Kᵢ' are plotted in Figure 3; Kᵢ' of group 1 equals Kᵢ. In contrast to Kᵢ, mean Kᵢ' in the four experimental groups was not less than that in group 1 for any brain region. During CPR in groups 2 and 5, mean Kᵢ' for the medulla was significantly greater than that in group 1.

Mean aortic blood pressure and arterial blood gases during the 2 minutes of AIB circulation are shown in Table 2. Immediately following defibrillation in group 3, aortic systolic blood pressure increased to a mean peak of 297±23 mm Hg. However, this peak was short-lived, and systolic pressure decreased to a mean of 216±18 mm Hg by 1 minute after the peak and 183±20 mm Hg by the next minute.

In the two dogs in which the venous outflow technique was used to elevate cerebral venous pressure (to 38 mm Hg in one and 75 mm Hg in the other), cerebral perfusion pressure averaged 27 and 45 mm Hg, respectively, during the 10 minutes following AIB injection. In these two dogs, Kᵢ was higher for all brain regions than in group 1 (Table 3).

**Discussion**

We found no direct evidence of increased BBB permeability to a small molecule such as AIB in any experimental protocol tested in this dog model of CPR. During CPR, there are large phasic increases in both arterial and jugular venous blood pressures, with peak sagittal sinus pressure reaching 30–40 mm Hg. Thus, we anticipated that postischemic disruption of the BBB would be enhanced by large fluctuations of cerebral venous pressure. The reason we did not find disruption may be related to the simultaneous increase in cerebrospinal fluid pressure...
during the chest compression phase of CPR.9 If these increases in sagittal sinus pressure and cerebrospinal fluid pressure were perfectly matched in amplitude and time phase, there would be no dynamic change in transmural pressure. We found that elevating the cerebral venous pressure without ischemia increased $K_i$, when the cerebrospinal fluid was drained concomitantly. Thus, increased cerebrospinal fluid pressure likely protects the BBB during chest compression.

Another factor may be the presence of venous hypercapnia. Baumbach et al10 showed less disruption of the BBB during hypertension accompanied by hypercapnia than during normocapnic hypertension. Whether the mechanism of this effect is due to attenuation of the increase in pial venular pressure by hypercapnia or to a direct effect on the BBB unrelated to venous pressure is unclear. Thus, it is possible that slow washout of CO$_2$ during CPR after complete ischemia exerts a protective effect on the BBB.

The period immediately following CPR is one during which the BBB is prone to disruption because of cerebral vasodilation in the presence of arterial hypertension. Aortic blood pressures of $\geq$160 mm Hg have been associated with BBB disruption,11 and the disruption is detectable with AIB.12 During CPR, Arai et al4 observed extravasation of Evans blue microscopically following defibrillation in eight of nine dogs in which the peak aortic systolic blood pressure averaged approximately 250 mm Hg. In our study, the transient increase in aortic systolic blood pressure to a mean of 297 mm Hg may not have lasted sufficiently long to cause disruption. One dog exhibited elevated $K_i$ in the brain stem and spinal cord but not the cerebral cortex. This regional pattern is unexpected because during hypertension the brain stem is more resistant to BBB disruption than the cerebral cortex.13 However, the $[3H]$inulin plasma-to-tissue ratio in this dog was increased relative to that in group 1. Thus, this increase in $K_i$ might have been due to only an increase in capillary surface area.

In other models of cerebral ischemia without the mechanical effects of CPR, two phases of increased BBB permeability are sometimes observed. With reversible middle cerebral artery occlusion, a biphasic pattern of disruption can occur, first 15 minutes and then 5 hours after ischemia.2 With global ischemia in rats, the BBB was disrupted only during the early stages following 15 or 30 minutes of ischemia, with recovery 24 hours after the original insult.14 With a longer ischemic period lasting 60 minutes,
We suggest that the decreased $K_j$ is due to decreased be expected to allow for substantial back-diffusion that it was lower than control for some brain regions. Increased $K_j$.

but it should not prevent the detection of an inhibition may affect the precise quantification of $K_j$, unless permeability were high in the first place, which is unlikely since $K_j$ remained low. Thus, transporter inhibition may affect the precise quantification of $K_j$, but it should not prevent the detection of an increased $K_j$.

We found not only that $K_j$ was not elevated, but that it was lower than control for some brain regions. We suggest that the decreased $K_j$ is due to decreased surface area of the capillary bed because brain plasma volume as measured by the [$^3H$]inulin space was decreased. Although it is possible that some plasma radiolabel was lost after death when the tissue was excised, it is unlikely that there was a systematic error that could account for the 30–40% reduction in plasma volume in the experimental groups compared with the control group. During CPR, cerebral blood flow is near normal in this model. Thus, if cerebral blood flow is normal and cerebral plasma volume is low, then complete capillary recruitment is unlikely during CPR after ischemia, and reperfusion will be heterogeneous. Kagstrom et al demonstrated the presence of reperfusion defects with normal cerebral perfusion pressure immediately after 15 minutes of ischemia in rats. With CPR, reperfusion heterogeneity may be even more severe because of subnormal cerebral perfusion pressure. Recently, Ennis et al observed a 20% decrease in the number of perfused capillaries in gerbils after 3 hours of incomplete ischemia and 11 minutes of reperfusion that partially accounted for the decreased $K_j$ for AIB.

Four hours after CPR, no increase in $K_j$ was noted. In parallel experiments, mean±SEM cerebral blood flow was only $18±1$ ml/min/100 g 4 hours after CPR, representing a 42% decrease from control values. The present [$^3H$]inulin tissue-to-plasma ratio was 40% lower than control. Thus, cerebral blood flow and plasma volume decreased equally during hypoperfusion. Similar results have been observed after ischemia in gerbils. If the cerebral blood volume is diminished, then $K_j$ may be decreased because of a decreased capillary surface area.

In conclusion, using a small amino acid to assess the integrity of the BBB in dogs after cardiac arrest, we found no substantial disruption of the BBB associated with chest compression during CPR, immediately following defibrillation, or 4 hours after defibrillation. Therefore, circulating hydrophilic substances such as epinephrine typically administered in high doses during CPR are unlikely to gain access to the vascular smooth muscle or to neurons, where they could indirectly affect cerebral blood flow or metabolism.

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References


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