Brain tissue oxygen monitoring identifies cortical hypoxia and thalamic hyperoxia after experimental cardiac arrest in rats

Mioara D. Manole[1,2], Patrick M. Kochanek[1–3], Hülya Bayır[1,3], Henry Alexander[1,3], Cameron Dezfulian[1–3], Ericka L. Fink[1,3], Michael J. Bell[1,3] and Robert S.B. Clark[1–3]

BACKGROUND: Optimization of cerebral oxygenation after pediatric cardiac arrest (CA) may reduce neurological damage associated with the post-CA syndrome. We hypothesized that important alterations in regional partial pressure of brain tissue oxygen (PbO_2) occur after resuscitation from CA and that clinically relevant interventions such as hyperoxia and blood pressure augmentation would influence PbO_2.

METHODS: Cortical and thalamic PbO_2 were monitored in immature rats subjected to asphyxial CA (9 or 12 min asphyxia) and sham-operated rats using oxygen sensors.

RESULTS: Thalamus and cortex showed similar baseline PbO_2. Postresuscitation, there was early and sustained cortical hypoxia in an insult-duration dependent fashion. In contrast, thalamic PbO_2 initially increased fourfold and afterwards returned to baseline values. PbO_2 level was dependent on the fraction of inspired O_2, and the response to oxygen was more pronounced after a 9 vs. 12 min CA. After a 12 min CA, PbO_2 was modestly affected by blood pressure augmentation using epinephrine in the thalamus but not in the cortex.

CONCLUSION: After asphyxial pediatric CA, there is marked regional variability of cerebral oxygenation. Cortical hypoxia is pronounced and appears early, whereas thalamic hyperoxia is followed by normoxia. Compromised PbO_2 in the cortex may represent a relevant and clinically measurable therapeutic target aimed at improving neurological outcome after pediatric CA.

Hypoxic ischemic brain injury affects 53–76% of children successfully resuscitated from cardiac arrest (CA) (1). Brain tissue hypoxia, cerebral hypoperfusion, and impaired autoregulation in the early post-CA syndrome have potential implications for secondary brain injury in patients successfully resuscitated from CA. The International Liaison Committee on Resuscitation emphasized in a recent statement that good neurological outcome is contingent upon optimal post-CA care and identified important knowledge gaps in (i) optimal oxygen delivery during the initial stages of reperfusion and (ii) optimal cerebral monitoring after CA (2). Early optimization of cerebral oxygen delivery to meet metabolic demands may reduce the neurological damage associated with post-CA brain injury.

After experimental pediatric asphyxial CA, cortical regions have decreased perfusion, while thalamic regions are hyperemic (3). Cortical hypoperfusion observed in our model may be associated with cortical hypoxia. Serial and regional partial pressure of brain tissue oxygen (PbO_2) after experimental asphyxial CA has not been characterized. Multiple studies have demonstrated that after traumatic brain injury, direct monitoring of PbO_2 is safe, and some studies have suggested that targeting specific thresholds for PbO_2 has been associated with improved outcomes compared with historical controls (4–8). Although brain tissue monitoring was included in the treatment guidelines for adult victims of traumatic brain injury in 2007, PbO_2 monitoring after pediatric CA is not used routinely.

Based on our previous studies of postresuscitation cerebral perfusion, we hypothesized that important differences in regional PbO_2 would be observed during early post-CA syndrome after experimental asphyxial CA and that clinically relevant interventions such as hyperoxia and blood pressure augmentation would influence PbO_2.

RESULTS

Regional PbO_2 During and After Asphyxial CA

Table 1 presents physiological data for the groups of rats that underwent CA. Mean arterial pressure (MAP) increased at 5–10 min postresuscitation, then decreased, and was lower than baseline after 30 min postresuscitation. The baseline MAP was lower in the 12 min asphyxial CA groups that underwent thalamic PbO_2 measurements vs. 9 min asphyxial CA that underwent cortical or thalamic PbO_2 measurements (55 ± 2 vs. 67 ± 2 and 72 ± 4 mm Hg, respectively; $P < 0.05$). $PaCO_2$ was maintained in the normal range at all time points except for an increase to 46 ± 1.6 mm Hg only at 60 min postresuscitation in the 9 min thalamic group. Hemoglobin decreased vs. baseline at 30, 60, and 120 min after 9 and 12 min CA, with no difference between groups at each time point.

[1]Safar Center for Resuscitation Research, University of Pittsburgh, Pittsburgh, Pennsylvania; [2]Department of Pediatrics, University of Pittsburgh, Pittsburgh, Pennsylvania; [3]Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania. Correspondence: Mioara D. Manole (mioara.manole@chp.edu)

Received 29 January 2013; accepted 4 July 2013; advance online publication 15 January 2014. doi:10.1038/pr.2013.220

Table 1. MAP, pH, PaO_2, $PaCO_2$, and Hb at baseline (immediately before asphyxia) and after asphyxial CA

			Baseline	10 min	30 min	60 min	120 min
Cortex	9 min	MAP	67 ± 2	78 ± 2*	56 ± 2*	52 ± 2*	52 ± 2*
		pH	7.40 ± 0.01	7.29 ± 0.01*	7.46 ± 0.02*	7.47 ± 0.01*	7.44 ± 0.03
		PaO_2	207 ± 11	402 ± 12*	412 ± 10*	183 ± 15*	235 ± 6
		$PaCO_2$	37 ± 1	41 ± 1	36 ± 1	37 ± 1	36 ± 4
		Hb	9.0 ± 0.2	8.9 ± 0.2	8.2 ± 0.2*	8.2 ± 0.5*	8.1 ± 0.3*
	12 min	MAP	65 ± 2	86 ± 3*	55 ± 5*	49 ± 3*	47 ± 3*
		pH	7.39 ± 0.01	7.27 ± 0.02*	7.40 ± 0.01	7.44 ± 0.01	7.39 ± 0.01
		PaO_2	212 ± 3	411 ± 20*	403 ± 1*	266 ± 38	208 ± 5
		$PaCO_2$	36 ± 1	39 ± 3	37 ± 2	37 ± 1	38 ± 1
		Hb	8.7 ± 0.3	8.9 ± 0.3	8.4 ± 0.2*	7.9 ± 0.3*	7.7 ± 0.3*
Thalamus	9 min	MAP	72 ± 4	73 ± 1	58 ± 2*	49 ± 3*	55 ± 2*
		pH	7.36 ± 0.10	7.32 ± 0.10*	7.37 ± 0.10*	7.34 ± 0.10*	7.42 ± 0.10*
		PaO_2	210 ± 7	302 ± 17*	360 ± 7*	204 ± 3	228 ± 7
		$PaCO_2$	37 ± 0.8	39 ± 2.5	39 ± 2.0	46 ± 1.6*	33 ± 2.5
		Hb	10 ± 0.2	9.6 ± 0.7	8.9 ± 0.2	8.0 ± 0.1*	8.5 ± 0.1
	12 min	MAP	55 ± 2	72 ± 4*	42 ± 5*	41 ± 2*	40 ± 1*
		pH	7.35 ± 0.01	7.25 ± 0.02	7.36 ± 0.01	7.39 ± 0.01	7.34 ± 0.01
		PaO_2	192 ± 11	385 ± 36*	375 ± 14*	184 ± 6	207 ± 5
		$PaCO_2$	41 ± 2	40 ± 4	38 ± 1	42 ± 1	44 ± 1
		Hb	9.1 ± 0.1	9.7 ± 0.2*	8.9 ± 0.2	8.1 ± 0.2*	7.7 ± 0.2*

CA, cardiac arrest; Hb, hemoglobin; MAP, mean arterial pressure.

*$P < 0.05$ vs. baseline.

Baseline PbO_2 was similar for cortical and thalamic areas. After the onset of asphyxia, PbO_2 decreased to less than 5 mm Hg within 60 s in both regions and in all rats. After cardiopulmonary resuscitation, we observed a rapid rise in PbO_2 in the thalamus and a more gradual rise in PbO_2 in the cortex.

Figure 1a illustrates cortical PbO_2 after resuscitation from 9 and 12 min asphyxial CA. After 9 min asphyxial CA, mean cortical PbO_2 was similar to baseline at 5, 10, and 15 min (64 ± 22, 39 ± 8, and 33 ± 6 mm Hg at 5, 10, and 15 min, respectively) and then lower than baseline from 30 min (19 ± 4 mm Hg) to 120 min (25 ± 5 mm Hg) postresuscitation. After 12 min asphyxial CA, cortical PbO_2 was similar to baseline at 5–15 min (43 ± 18, 25 ± 7, and 25 ± 6 mm Hg) and then lower than baseline from 30 min (15 ± 4 mm Hg) to 120 min (9 ± 2 mm Hg) postresuscitation (**Figure 1a**). Cortical PbO_2 was lower in rats subjected to 12 min asphyxial CA vs. 9 min asphyxial CA at 120 min postresuscitation ($P < 0.05$).

Figure 1b illustrates thalamic PbO_2 after resuscitation from 9 and 12 min asphyxial CA. After both 9 and 12 min asphyxial CA, thalamic PbO_2 was markedly increased vs. baseline at 5 min (281 ± 25 vs. 66 ± 16.3, and 256 ± 45 vs. 63 ± 6 mm Hg; $P < 0.05$ after 9 and 12 min asphyxia, respectively) and remained elevated at 10 min after resuscitation (151 ± 23 and 217 ± 50 mm Hg; $P < 0.05$ vs. baseline after 9 and 12 min asphyxia, respectively). After the initial increase, thalamic PbO_2 decreased toward baseline for the duration of monitoring and remained well above 20 mm Hg.

Post-CA Response of PbO_2 to Supplemental Oxygen

The response to supplemental oxygen was evaluated at 120 min after resuscitation from 9 or 12 min CA. At 120 min after resuscitation, the rats receive a fraction of inspired oxygen (FiO_2) = 0.5 in our model. At that time point, we decreased the FiO_2 to 0.21 and gradually increased the FiO_2 to 1.0. Decreasing the FiO_2 to 0.21 at 120 min after CA resulted in arterial hypoxemia (transcutaneous oxygen saturation, $SatO_2$ = 83 ± 3%, PaO_2 = 46.2 ± 4.2 mm Hg). At an FiO_2 of 0.21, cortical PbO_2 was 16 ± 2 and 5 ± 2 mm Hg after 9 and 12 min asphyxial CA, respectively, whereas thalamic PbO_2 was 19 ± 5 and 30 ± 3 mm Hg after 9 and 12 min asphyxial CA, respectively (**Figure 2**).

Cortical and thalamic PbO_2 and $SatO_2$ values for individual FiO_2 concentrations are shown in **Figure 2**. Increasing FiO_2 from 0.21 to 0.3 resolved the observed hypoxemia and increased the $SatO_2$ above 95% in all rats. Further increases in FiO_2 increased $SatO_2$ to 99–100%. Under these conditions, cortical and thalamic PbO_2 had different responses to increased FiO_2. Cortical PbO_2 after 9 min of asphyxia increased progressively with supplemental oxygen from FiO_2 = 0.21 to FiO_2 = 0.4 ($P < 0.05$). Further increase in FiO_2 to 0.5 and 1 did not result in further increase in cortical PbO_2 after 9 min of asphyxia. After 12 min of asphyxia, cortical PbO_2 values were 7.1 ± 3 and 8.3 ± 3 mm Hg at FiO_2 = 0.3 and 0.4, respectively (P = 0.3 and 0.2, FiO_2 = 0.3 and 0.4 vs. FiO_2 = 0.21, respectively). PbO_2 was slightly increased at FiO_2 = 0.5 and 1 (PbO_2 = 10.5 ± 8 mm Hg; $P < 0.05$ for FiO_2 = 0.5 vs. FiO_2 = 0.21).

Thalamic PbO_2 gradually increased with increases in supplemental oxygen after both 9 and 12 min of asphyxia (**Figure 2**).

Post-CA Response of PbO_2 to Epinephrine Infusion

To determine whether low cortical PbO_2 levels after 12 min asphyxial CA were related to low MAP, we administered

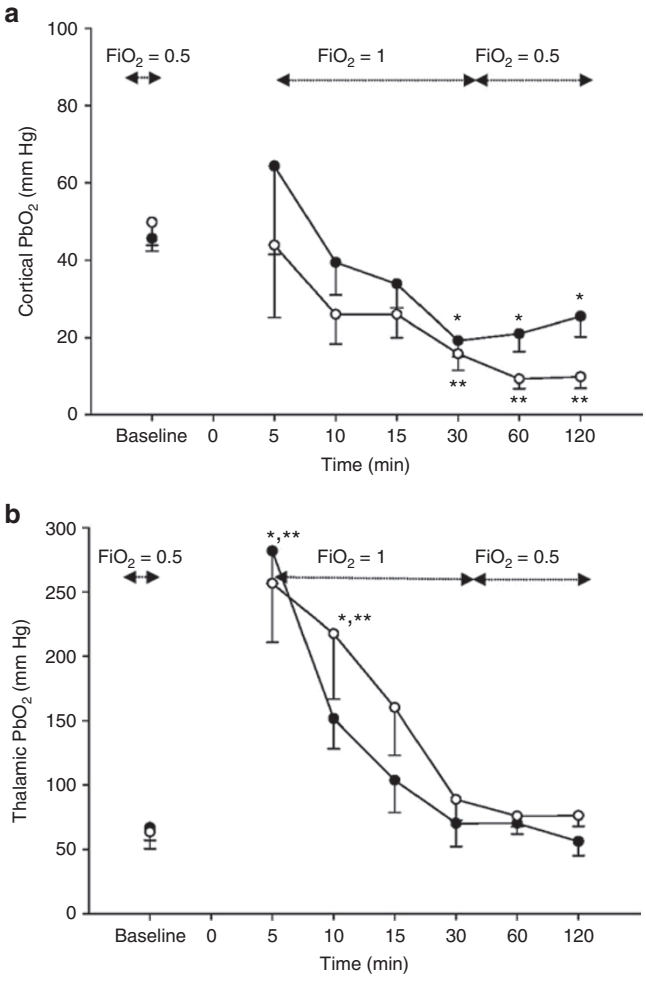

Figure 1. Partial pressure of brain tissue oxygen (PbO_2) after resuscitation from cardiac arrest (CA). (**a**) Cortical and (**b**) thalamic PbO_2 at baseline and after resuscitation from 9 and 12 min of asphyxia. Time 0 represents the time of CA. Black circles represent 9 min CA group and white circles represent 12-min CA group. $n = 6$ per group for thalamic PbO_2 measurements and $n = 12$ per group for cortical PbO_2 measurements. $^*P < 0.05$ vs. baseline for 9 min insult. $^{**}P < 0.05$ vs. baseline for 12 min insult. FiO_2, fraction of inspired oxygen.

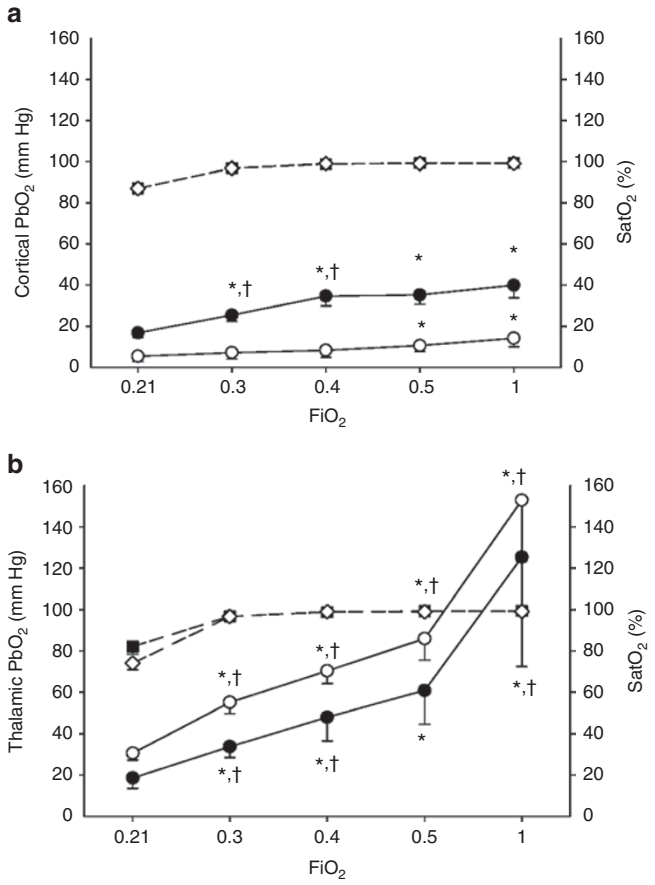

Figure 2. Post-cardiac arrest (CA) response of the partial pressure of brain tissue oxygen (PbO_2) to supplemental oxygen. (**a**) Cortical and (**b**) thalamic partial PbO_2 and $SatO_2$ response to increase in FiO_2 from 0.21 to 0.3, 0.4, 0.5, and 1.0 after 9 and 12 min asphyxia. Black circles represent PbO_2 for the 9 min CA group, white circles represent PbO_2 for the 12 min CA group, black squares represent $SatO_2$ for the 9 min CA group, and white diamonds represent $SatO_2$ for the 12 min CA group. $n = 6$ per group per region. $^*P < 0.05$ vs. $FiO_2 = 0.21$. $^\dagger P < 0.05$ vs. previous FiO_2. FiO_2, fraction of inspired oxygen; PbO_2, pressure of brain tissue oxygen; $SatO_2$, transcutaneous oxygen saturation.

epinephrine to increase MAP at 120 min after CA. A 2 mcg kg^{-1} min^{-1} epinephrine infusion increased MAP from 47 ± 3.7 to 66.6 ± 6.8 mm Hg (baseline MAP), and further infusion of epinephrine to 20 mcg kg^{-1} min^{-1} increased MAP to 82.3 ± 5.8 mm Hg (above baseline MAP). Cortical PbO_2 did not increase with epinephrine infusion (**Figure 3**). Conversely, thalamic PbO_2 increased during highdose epinephrine infusion ($P < 0.05$, epinephrine 20 mcg kg^{-1} min^{-1} vs. before epinephrine infusion, **Figure 3**). In sham rats treated with epinephrine infusion, we observed a similar pattern of PbO_2 response: no increase in cortical PbO_2 and increase in thalamic PbO_2 at 4, 10, and 20 mcg kg^{-1} min^{-1} (data not shown).

DISCUSSION

To our knowledge, this is the first study reporting direct measurements of PbO_2 in a model of pediatric asphyxial CA. Our data suggest important region- and insult duration-dependent alterations in PbO_2 after CA. Cortical PbO_2 was reduced in the first 2 h after resuscitation, especially after longer insults, reaching thresholds consistent with cerebral ischemia (9). In distinct contrast, thalamic PbO_2 was markedly increased initially after CA and then returned to baseline. PbO_2 response to supplemental oxygen was also insult duration and region dependent.

PbO_2 reflects the local oxygen concentration in brain tissue and serves as a marker of the balance between regional oxygen supply and consumption. It is influenced by cerebral blood flow (CBF), MAP, PaO_2, hemoglobin concentration, and factors affecting diffusion of oxygen in brain (10). In clinical studies, PbO_2 values less than 10 mm Hg are associated with higher risk for ischemic brain injury (11,12) and with unfavorable outcome after traumatic brain injury (8). Similar threshold values have not been reported in either experimental or clinical CA.

Post-CA cortical hypoxia in this study reached levels that are below the accepted PbO_2 ischemic thresholds of 20 (5) or even 10 mm Hg in humans (8,11). Human data demonstrated

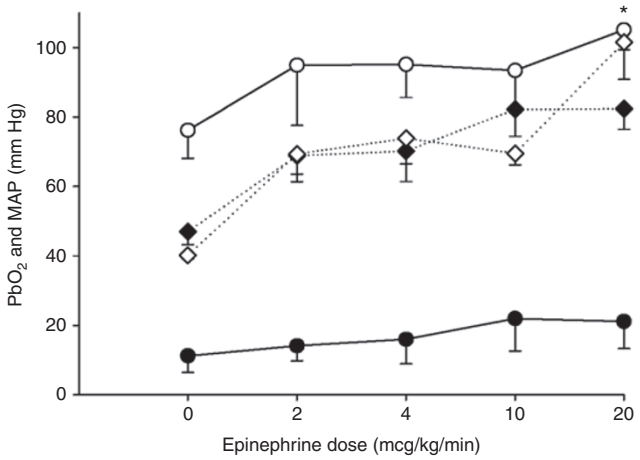

Figure 3. Cortical partial pressure of brain tissue oxygen (PbO_2) at 120 min after resuscitation from cardiac arrest (CA) (12 min asphyxia), before epinephrine (epinephrine dose = 0), and after infusion of epinephrine at doses of 2, 4, 10, and 20 mcg kg^{-1} min^{-1}. Black circles represent cortical PbO_2, white circles represent thalamic PbO_2, black diamonds represent mean arterial pressure (MAP) for the cortical group, and white diamonds represent MAP for the thalamic group. $n = 6$ per group. *$P < 0.05$ vs. before epinephrine.

ongoing ischemia and anaerobic metabolism in the cortex during the first 10 h and up to 40 h after CA (13,14). Similar to our study, in an experimental model of global cerebral ischemia produced by neck tourniquet in adult primates, PbO_2 was region dependent with cortical hypoxia starting at 1 h post-ischemia (15). Conversely, upon resuscitation from neonatal hypoxia with brief (1 min) CA, Linner et al. (16) observed initial cortical hyperoxia followed by return to baseline levels in newborn piglets. This is consistent with the hypothesis that PbO_2 changes are insult-duration dependent, with shorter durations of CA producing less hypoxia, similar to our previously described insult-duration dependency of CBF post-CA (3). Age-related differences or species differences could also be involved. If this observation translates to children resuscitated from out-of-hospital CA, where the duration of no-flow is most often unknown, PbO_2 could have potential utility in insult-duration stratification and prognostication.

Our data suggest that PbO_2 parallels CBF in our model. Post-CA cortical hypoxia mirrors the cortical hypoperfusion we previously observed, whereas thalamic hyperoxia parallels thalamic hyperemia (3). Isoflurane, shown to preferentially increase subcortical CBF (17–20), increased thalamic PbO_2 in our sham rats while having no effect on cortical PbO_2 (pilot data, not shown). The association between PbO_2 and CBF was also reported in noninjured rats (21) and models of global ischemia (22). These observations suggest that continuous PbO_2 monitoring may be used as a surrogate marker for cerebral perfusion early after CA.

Supplemental oxygen increased thalamic and cortical PbO_2, with a more robust effect after 9 vs. 12 min CA. It was previously reported that the response of PbO_2 to hyperoxia is diminished in pathological compared with normal tissue (23–25), and increased supplemental oxygen does not correspond to an improvement in brain oxygen delivery in areas with severely reduced CBF (26). We previously reported that CBF is decreased in cortex, more pronounced after prolonged insults, and increased in thalamus after CA in our model (3). Our findings are in accordance with the hypothesis that raising FiO_2 in the injured brain yields an increase in PbO_2 but is highly dependent on adequate CBF. These observations suggest that interventions targeted to increase PbO_2 after long durations of CA should primarily focus on increasing CBF. Further studies of strategies to increase cortical PbO_2 via blood flow promoting vasodilatatory agents are warranted. Possible CBF promoting strategies include endothelin receptor antagonists (27), nitrite (28), or inhibition of 20-HETE (29), among other therapies. In our study, conditions of mild arterial hypoxemia, on the contrary, resulted in lower PbO_2 in shams and post-CA, suggesting that arterial hypoxemia should be avoided post-CA.

Increasing MAP using epinephrine infusion after long insults did not significantly increase cortical PbO_2, although it increased thalamic PbO_2 at high doses only. This suggests that nonspecific interventions such as MAP augmentation may not be sufficient to increase PbO_2 in areas of cerebral hypoxia after CA. Our results also indicate that epinephrine could compromise cerebral microcirculatory flow after CA, similar to its effects in a piglet model of CA where despite increase in cerebral perfusion pressure after epinephrine infusion, microcirculatory blood flow decreased (30). As epinephrine is frequently used clinically postresuscitation from CA to stabilize hemodynamic parameters, it would be important to determine if other strategies to raise MAP might be able to improve PbO_2 and microcirculatory flow. While MAP was measured during the epinephrine infusion, measurement of the intracranial pressure to assess the cerebral perfusion pressure could have given us additional information regarding the cerebrovascular effect of epinephrine.

We chose to study the effect on PbO_2 of two clinically relevant and frequently used therapies during post-CA syndrome: oxygen and epinephrine. Other clinical strategies to augment PbO_2, such as blood transfusion, administration of pentobarbital or controlled hypoventilation, not studied here, might have some utility.

We found some degree of variability of PbO_2 in our study, both in shams and injured rats. This variability was observed in other studies (15) and is consistent with data showing that cortical PbO_2 varies with as much as 20 mm Hg depending on the cortical area and layers of the somatosensory cortex (31). Likewise, hippocampal PbO_2 varies within different areas (32). Our data suggest, however, that marked reductions of PbO_2 in the cortex are observed after CA and represent a potential therapeutic target for postresuscitation approaches designed to raise PbO_2.

The ratio of PbO_2/PaO_2 followed the same pattern as PbO_2 (data not shown), confirming that the region-specific variability to PbO_2 in response to CA is not a function of changes in FiO_2. We found some variability in our physiological data. The thalamic group with lower MAP (12 min asphyxial CA) had a similar PbO_2 tracing with the 9 min thalamic group, and thus MAP values were unlikely to have affected our results. We

also observed a decrease in hemoglobin of 0.9–1.5 mg/dl over time in all groups due to blood sampling, with no difference between groups. In our model, we observe a similar decrease in hemoglobin in sham rats with no change in PbO_2, suggesting that this degree of decrease in hemoglobin does not affect PbO_2 in sham or injured rats.

Relevant to the current study, in previous studies, we did not observe sex-based differences in postarrest CBF (3), although we observed a sex-based difference in response to a specific treatment (polynitroxyl albumin) (33). As one aspect of the current study was to evaluate PbO_2 as a potential surrogate for continuous CBF measurements after CA, we chose to limit the experiments to males. Future studies using PbO_2, particularly those evaluating treatments, should include males and females.

In this model of pediatric asphyxial CA, selective neuronal death in cortex, thalamus, hippocampus, and cerebellum is seen, along with behavioral deficits (34,35). However, the relationship between cortical PbO_2 and histopathological and functional outcome in our model remains to be determined. The threshold for cortical hypoxia that produces neuronal injury may even be lower than 10 mm Hg and remains uncertain. Age-related differences in the PbO_2 threshold for hypoxia after CA also remain undefined. An intriguing hypothesis is that postischemic thalamic hyperoxia might also be detrimental. Further experiments in our model assessing the PbO_2 threshold and whether goal-directed PbO_2 therapy improves outcome are warranted.

In conclusion, PbO_2 after asphyxial CA in immature rats is region dependent: there is cortical hypoxia but early thalamic hyperoxia. These findings mimic the pattern of CBF in this model, suggesting that PbO_2 is representative of oxygen delivery and CBF. Increasing supplemental oxygen improves cortical hypoxia. If our model translates to the human condition, these data suggest a possible utility for PbO_2 monitoring after CA in children. Further studies in this model are warranted to assess if goal directed therapy targeting cerebral oxygenation improves outcome after experimental pediatric asphyxial CA.

METHODS

Studies were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh, and the care and handling of the animals were in accord with the National Institutes of Health (Bethesda, MD) guidelines. We used male postnatal day 16–18 Sprague-Dawley rats (30–46 g; $n = 56$). We measured PbO_2 after CA in the cortex and thalamus in separate groups of rats after asphyxial CA and the response of PbO_2 to hyperoxia or MAP augmentation after CA.

Anesthesia and Surgery

Rats were initially anesthetized with 3% isoflurane/50% N_2O/balance O_2 in a Plexiglas chamber (Henry Schein, US) until unconscious and then their tracheas were intubated with an 18-gauge angio catheter (Becton, Dickinson and Company, US). Mechanical ventilation was started and ventilatory rates and tidal volumes were adjusted to maintain $PaCO_2$ at 35–45 mm Hg. Femoral arterial and venous catheters (PE 10) were inserted to monitor MAP and infuse medications. During surgery, anesthesia was maintained with 1.5% isoflurane/50% N_2O/balance O_2. Isoflurane was discontinued after the placement of arterial and venous catheters, and anesthesia was maintained with fentanyl as described below. MAP and heart rate were continuously monitored. Rectal temperature was maintained at 37 °C using a heated water blanket.

PbO_2 Electrode Placement

The head was stabilized in a stereotaxic instrument using ear bars. A small burr hole (2 mm) was drilled in the skull 2 mm lateral and 3.3 mm posterior to bregma. PbO_2 was measured continuously using a Clark type tissue electrode (Ox-50, Unisense, Denmark) inserted at either a depth of 1 mm for cortical PbO_2 measurements or at a depth of 6 mm for thalamic PbO_2 measurements. We chose to measure PbO_2 in the cortex and thalamus because these are brain regions with the lowest or highest postresuscitation CBF, respectively, in our previous report (3). The location of the electrode in the cortex or thalamus was verified in brain sections after injection of Evans Blue (Sigma Aldrich, US) at the conclusion of the experiment. Brain temperature was continuously monitored with an intraparenchymal sensor. Three groups (cortical 9 and 12 min groups and thalamic 9 min groups) had mean brain temperatures between 36.2 °C and 36.9 °C. Due to an experimental oversight, the thalamic 12-min group did not have brain temperature monitored; however, the body temperature, which parallels the brain temperature in our model, was maintained constant in this group. Baseline parameters for MAP, PaO_2, pH, $PaCO_2$, and PbO_2 were obtained immediately before the asphyxial insult.

PbO_2 Measurements in Shams Under Different Anesthetic Conditions

In pilot experiments, we determined the effects of two anesthetics: isoflurane and fentanyl on cortical and thalamic PbO_2 in sham rats. Cortical PbO_2 was measured in one group and thalamic PbO_2 in the other ($n = 8$, 4 per group). Each group initially was anesthetized with isoflurane, and PbO_2 was measured after a 10-min stabilization period. Isoflurane was then discontinued, and a fentanyl infusion at 50 μg kg^{-1} h^{-1} was started. The rats were observed for 30 min prior to measuring the PbO_2 to assure wash out of isoflurane. Cortical PbO_2 values were similar using isoflurane or fentanyl anesthesia. Thalamic PbO_2 were higher using isoflurane vs. fentanyl anesthesia (109 ± 32% increase in thalamic PbO_2 using isoflurane vs. fentanyl; $P < 0.05$). Therefore, in all CA experiments, anesthesia was maintained with fentanyl infusion to minimize effects of anesthesia on PbO_2.

Asphyxial CA

We used an established asphyxial CA protocol (3). Rats received intravenous fentanyl infusion at 50 μg kg^{-1} h^{-1} to provide anesthesia and vecuronium infusion at 5 mg kg^{-1} h^{-1} to induce neuromuscular blockade. We used fentanyl as the anesthetic agent during the CA experiments because it is clinically relevant, and unlike the inhaled anesthetics, it does not affect CBF (19). The FiO_2 was reduced from 0.5 to 0.21 for 1 min before asphyxia to avoid preinsult hyperoxygenation. The tracheal tube was disconnected from the ventilator for 9 or 12 min. Resuscitation was started by reconnecting the ventilator and reinstituting mechanical ventilation at an FiO_2 of 1.0. Epinephrine (0.005 mg kg^{-1}) and sodium bicarbonate (1 mEq kg^{-1}) were administered intravenously, followed by manual chest compressions until return of spontaneous circulation. The fentanyl infusion was restarted 30 min after resuscitation at the prearrest infusion rate. At 30 min after resuscitation, FiO_2 was decreased to 0.5. This oxygenation sequence during cardiopulmonary resuscitation and postresuscitation is consistent with common clinical practice at our institution. Arterial blood gas measurements were obtained at the time of arterial catheter insertion, at 30 and 60 min after CA and at the end of the experiment, and the ventilatory rates and tidal volumes were adjusted to a target of $PaCO_2$ of 35–45 mm Hg. $SatO_2$ was measured continuously with a pulse oximeter (MouseOx, Starr Life Sciences).

PbO_2 Measurements During and After CA

In separate groups of rats, cortical or thalamic PbO_2 were measured continuously before, during, and after 9 or 12 min asphyxial CA ($n = 6$ per group for thalamic PbO_2 measurements and $n = 12$ per group for cortical PbO_2 measurements). PbO_2 was recorded for analysis at baseline and at the following time points postresuscitation: 5, 10, 15, 30, 60, and 120 min. At the completion of 120-min postresuscitation period, these rats underwent PbO_2 measurements to assess the response to supplemental oxygen or epinephrine as described below.

Post-CA Response of PbO_2 to Supplemental Oxygen

We assessed cortical and thalamic PbO_2 responses to a gradual increase in FiO_2 from 0.21 to 1.0 (FiO_2 = 0.21, 0.3, 0.4, 0.5, and 1.0) at 120 min after resuscitation from 9 or 12 min asphyxial CA (n = 6 per group per region). According to our asphyxial CA protocol, rats receive FiO_2 = 1 at resuscitation and during the first 30 min postresuscitation. From 30–120 min after resuscitation, the rats receive an FiO_2 of 0.5. At 120 min after resuscitation, we decreased the FiO_2 to 0.21 and then gradually increased the FiO_2 as above. At each FiO_2, we recorded the $SatO_2$, PbO_2, and MAP after a 10-min stabilization period for PbO_2.

Post-CA Response of PbO_2 to Epinephrine

We assessed the post-CA cortical and thalamic PbO_2 response to an increase in MAP initially to baseline values, followed by MAP increase above baseline (n = 6 per group). At 120 min, after 12 min asphyxia, we increased MAP via infusion of epinephrine. We started at a dose of 2 mcg kg^{-1} min^{-1} to achieve MAP equal to baseline and then increased the infusion to 4, 10, and 20 mcg kg^{-1} min^{-1} (3,36) while the FiO_2 was maintained at 0.5. We administered the same infusion of epinephrine to sham rats (n = 6 per group) and measured PbO_2.

Statistical Analysis

Data were analyzed with the statistical software Systat, Sigmastat 11.2 (Systat Software, Chicago, IL). Data were expressed as mean ± SEM. A P value <0.05 was considered significant. We used repeated measures ANOVA with Student–Newman–Keuls *post-hoc* test to compare MAP, $PaCO_2$, PaO_2, pH, and PbO_2 values at each time point and within each group over time. For data that failed equal variance and normality, we ranked the data and afterwards performed repeated measures ANOVA.

STATEMENT OF FINANCIAL SUPPORT

This study was supported by the National Institutes of Health (NIH; Bethesda, MD) grant K08 HD058798 (to M.D.M.), NIH grant R01 HD075760 (to M.D.M.), American Heart Association grant 10BGIA3580040 (to M.D.M.), Children's Hospital of Pittsburgh Research Grant (Pittsburgh, PA; to M.D.M.), NIH grant R01 HD045968 (to R.S.B.C.), and NIH grant K23 NS065132 (to E.L.F.).

REFERENCES

1. Moler FW, Meert K, Donaldson AE, et al.; Pediatric Emergency Care Applied Research Network. In-hospital versus out-of-hospital pediatric cardiac arrest: a multicenter cohort study. Crit Care Med 2009;37:2259–67.
2. Nolan JP, Neumar RW, Adrie C, et al. Post-cardiac arrest syndrome: epidemiology, pathophysiology, treatment, and prognostication. A Scientific Statement from the International Liaison Committee on Resuscitation; the American Heart Association Emergency Cardiovascular Care Committee; the Council on Cardiovascular Surgery and Anesthesia; the Council on Cardiopulmonary, Perioperative, and Critical Care; the Council on Clinical Cardiology; the Council on Stroke. Resuscitation 2008;79:350–79.
3. Manole MD, Foley LM, Hitchens TK, et al. Magnetic resonance imaging assessment of regional cerebral blood flow after asphyxial cardiac arrest in immature rats. J Cereb Blood Flow Metab 2009;29:197–205.
4. Stiefel MF, Udoetuk JD, Storm PB, et al. Brain tissue oxygen monitoring in pediatric patients with severe traumatic brain injury. J Neurosurg 2006;105:Suppl:281–6.
5. Narotam PK, Morrison JF, Nathoo N. Brain tissue oxygen monitoring in traumatic brain injury and major trauma: outcome analysis of a brain tissue oxygen-directed therapy. J Neurosurg 2009;111:672–82.
6. McCarthy MC, Moncrief H, Sands JM, et al. Neurologic outcomes with cerebral oxygen monitoring in traumatic brain injury. Surgery 2009;146:585–90; discussion 590–1.
7. Lang EW, Mulvey JM, Mudaliar Y, Dorsch NW. Direct cerebral oxygenation monitoring–a systematic review of recent publications. Neurosurg Rev 2007;30:99–106; discussion 106–7.
8. Maloney-Wilensky E, Gracias V, Itkin A, et al. Brain tissue oxygen and outcome after severe traumatic brain injury: a systematic review. Crit Care Med 2009;37:2057–63.
9. Vespa PM. The implications of cerebral ischemia and metabolic dysfunction for treatment strategies in neurointensive care. Curr Opin Crit Care 2006;12:119–23.
10. Maloney-Wilensky E, Le Roux P. The physiology behind direct brain oxygen monitors and practical aspects of their use. Childs Nerv Syst 2010;26:419–30.
11. Goodman JC, Valadka AB, Gopinath SP, Uzura M, Robertson CS. Extracellular lactate and glucose alterations in the brain after head injury measured by microdialysis. Crit Care Med 1999;27:1965–73.
12. Kiening KL, Unterberg AW, Bardt TF, Schneider GH, Lanksch WR. Monitoring of cerebral oxygenation in patients with severe head injuries: brain tissue PO2 versus jugular vein oxygen saturation. J Neurosurg 1996;85:751–7.
13. Nordmark J, Rubertsson S, Mörtberg E, Nilsson P, Enblad P. Intracerebral monitoring in comatose patients treated with hypothermia after a cardiac arrest. Acta Anaesthesiol Scand 2009;53:289–98.
14. Bauer R, Gabl M, Obwegeser A, Galiano K, Barbach J, Mohsenipour I. Neurochemical monitoring using intracerebral microdialysis during cardiac resuscitation. Intensive Care Med 2004;30:159–61.
15. Nemoto EM, Erdmann W, Strong E, Rao GR, Moossy J. Regional brain PO2 after global ischemia in monkeys: evidence for regional differences in critical perfusion pressures. Stroke 1979;10:44–52.
16. Linner R, Werner O, Perez-de-Sa V, Cunha-Goncalves D. Circulatory recovery is as fast with air ventilation as with 100% oxygen after asphyxia-induced cardiac arrest in piglets. Pediatr Res 2009;66:391–4.
17. Farber NE, Harkin CP, Niedfeldt J, Hudetz AG, Kampine JP, Schmeling WT. Region-specific and agent-specific dilation of intracerebral microvessels by volatile anesthetics in rat brain slices. Anesthesiology 1997;87:1191–8.
18. Hansen TD, Warner DS, Todd MM, Vust LJ, Trawick DC. Distribution of cerebral blood flow during halothane versus isoflurane anesthesia in rats. Anesthesiology 1988;69:332–7.
19. Hendrich KS, Kochanek PM, Melick JA, et al. Cerebral perfusion during anesthesia with fentanyl, isoflurane, or pentobarbital in normal rats studied by arterial spin-labeled MRI. Magn Reson Med 2001;46:202–6.
20. Loeb AL, Raj NR, Longnecker DE. Cerebellar nitric oxide is increased during isoflurane anesthesia compared to halothane anesthesia: a microdialysis study in rats. Anesthesiology 1998;89:723–30.
21. Lowry JP, Boutelle MG, Fillenz M. Measurement of brain tissue oxygen at a carbon past electrode can serve as an index of increases in regional cerebral blood flow. J Neurosci Methods 1997;71:177–82.
22. Rossi S, Balestreri M, Spagnoli D, et al. Oxygen delivery and oxygen tension in cerebral tissue during global cerebral ischaemia: a swine model. Acta Neurochir Suppl 2000;76:199–202.
23. Longhi L, Valeriani V, Rossi S, De Marchi M, Egidi M, Stocchetti N. Effects of hyperoxia on brain tissue oxygen tension in cerebral focal lesions. Acta Neurochir Suppl 2002;81:315–7.
24. Hlatky R, Valadka AB, Gopinath SP, Robertson CS. Brain tissue oxygen tension response to induced hyperoxia reduced in hypoperfused brain. J Neurosurg 2008;108:53–8.
25. Meixensberger J, Dings J, Kuhnigk H, Roosen K. Studies of tissue PO2 in normal and pathological human brain cortex. Acta Neurochir Suppl (Wien) 1993;59:58–63.
26. Rossi S, Longhi L, Balestreri M, Spagnoli D, deLeo A, Stocchetti N. Brain oxygen tension during hyperoxia in a swine model of cerebral ischaemia. Acta Neurochir Suppl 2000;76:243–5.
27. Armstead WM. Age dependent endothelin contribution to NOC/oFQ induced impairment of NMDA cerebrovasodilation after brain injury. Peptides 2001;22:39–46.
28. Jung KH, Chu K, Ko SY, et al. Early intravenous infusion of sodium nitrite protects brain against *in vivo* ischemia-reperfusion injury. Stroke 2006;37:2744–50.
29. Poloyac SM, Zhang Y, Bies RR, Kochanek PM, Graham SH. Protective effect of the 20-HETE inhibitor HET0016 on brain damage after temporary focal ischemia. J Cereb Blood Flow Metab 2006;26:1551–61.
30. Ristagno G, Tang W, Huang L, et al. Epinephrine reduces cerebral perfusion during cardiopulmonary resuscitation. Crit Care Med 2009;37:1408–15.

31. Masamoto K, Takizawa N, Kobayashi H, Oka K, Tanishita K. Dual responses of tissue partial pressure of oxygen after functional stimulation in rat somatosensory cortex. Brain Res 2003;979:104–13.
32. Andreasen A, Danscher G, Juhl S, et al. Distinct differences in partial oxygen pressure at micrometer ranges in the rat hippocampal region. J Neurosci Methods 1997;72:15–21.
33. Manole MD, Kochanek PM, Foley LM, et al. Polynitroxyl albumin and albumin therapy after pediatric asphyxial cardiac arrest: effects on cerebral blood flow and neurologic outcome. J Cereb Blood Flow Metab 2012;32:560–9.
34. Fink EL, Alexander H, Marco CD, et al. Experimental model of pediatric asphyxial cardiopulmonary arrest in rats. Pediatr Crit Care Med 2004;5:139–44.
35. Shoykhet M, Simons DJ, Kochanek PM, Clark RSB Anatomic evidence of injury to thalamocortical structures in a rodent model of pediatric asphyxia cardiac arrest. Critical Care Med 2011;38:Suppl:A152.
36. Dewachter P, Raëth-Fries I, Jouan-Hureaux V, et al. A comparison of epinephrine only, arginine vasopressin only, and epinephrine followed by arginine vasopressin on the survival rate in a rat model of anaphylactic shock. Anesthesiology 2007;106:977–83.