Blood to Brain Transport after Newborn Cerebral Ischemia/Reperfusion Injury (43254)

ROBERT MIRRO,*,†,‡,[1] WILLIAM M. ARMSTEAD,* DAVID W. BUSIJA,* AND CHARLES W. LEFFLER*,†

Departments of Physiology and Biophysics, Pediatrics,† and Obstetrics and Gynecology,‡ Laboratory for Research in Neonatal Physiology, The University of Tennessee, Memphis, Tennessee 38163*

Abstract. **These experiments examine the transfer of urea, sodium, and sucrose from blood to brain in an animal model of newborn cerebral ischemia-reperfusion injury. Cerebral ischemia (20 min) was produced in anesthetized, ventilated piglets by increasing intracranial pressure above mean arterial blood pressure, thereby reducing cerebral perfusion pressure to zero. The blood to brain transfer of urea, sodium, and sucrose was then measured in sham control piglets and at 30 min and 2 hr of reperfusion following ischemia. At 30 min of reperfusion, urea and sodium transfer were increased while sucrose transfer was unchanged. However, at 2 hr of reperfusion, transfer of all three tracers was elevated. The difference in the time course of increased transport of these substances into the brain following ischemia cannot be explained by size differences, indicating that changes in the blood-brain barrier following ischemia are more complex than merely opening junctions between cells and creating leaky sites. Alterations in blood-brain barrier transport could participate in altered neuronal function after ischemia-reperfusion injury.** [P.S.E.B.M. 1991, Vol 197]

Cerebral capillary endothelial cells provide a barrier which protects the brain parenchyma from hormones, neurotransmitters, and ions that could adversely affect nerve cell function. The endothelial cells are not only bound tightly together to form an anatomical barrier, but also contain selective transport and enzymatic mechanisms that regulate the movement of substances between blood and brain. In newborns, the maturity and function of the blood-brain barrier are important variables. An effective blood-brain barrier exists at birth in man, sheep, guinea pig, and pig (1). Functionally, however, the blood-brain barrier of the newborn is different from that of the adult with properties that result in the transport of different substances at differing rates (2). The concept of a specialized newborn blood-brain barrier is reasonable given the unique substrate needs of the developing brain.

Ischemia-reperfusion injury significantly alters cerebral hemodynamics. In adults of several species, an initial hyperemia followed by a reduction in cerebral blood flow has been observed following cerebral ischemia (3). For newborn pigs, however, a different pattern of reperfusion has been observed in which the hyperemic response in the cerebrum is far less in magnitude and duration than in the rest of the brain (4). Microvascular responses following newborn cerebral ischemia-reperfusion are also affected, including a selective loss of prostanoid-dependent dilation (5). The specialized transport properties of the newborn blood-brain barrier coupled with the altered vascular responsiveness observed after ischemia-reperfusion injury led us to study the transport from blood to brain in a model of newborn cerebral ischemia reperfusion injury.

Materials and Methods

Newborn pigs (1.5–2.2 kg, 1–5 days old) anesthetized with halothane and nitrous oxide were used in these experiments. Animals were ventilated with a positive pressure infant respirator after intubation with a 3.0-mm (i.d.) endotracheal tube. Body temperature was maintained at 37–38°C with an overhead radiant heater. Catheters were placed in both femoral arteries and femoral veins.

Cerebral Ischemia-Reperfusion Injury. Cerebral ischemia-reperfusion injury was produced using a hollow stainless steel bolt that was implanted in the right parietal cranium (5). An incision was made in the scalp and, using an electric drill with a hollow toothless bit, a 3-mm piece of skull was removed without damaging

[1] To whom correspondence and requests for reprints should be addressed at Newborn Center, 853 Jefferson Avenue, Room 201, Memphis, TN 38163.

Received December 17, 1990. [P.S.E.B.M. 1991, Vol 197]
Accepted March 5, 1991.

0037-9727/91/1973-0268$3.00/0

the dura. A hollow threaded bolt was screwed into the hole to a depth flush with the inner skull surface and the bolt was sealed in place with dental acrylic. Total brain ischemia was produced by reducing cerebral perfusion pressure to zero for 20 min. Artificial cerebrospinal fluid was infused through the bolt in order to raise the intracranial pressure (measured at the bolt via a sidearm manometer) to 15 mm Hg greater than mean arterial blood pressure. To prevent the arterial blood pressure from rising inordinately (Cushing response), blood was withdrawn as necessary to maintain mean arterial pressure no higher than 100 mm Hg. As the Cushing response subsided, the withdrawn blood (anticoagulated with citrate and dextrose) was reinfused. At the end of the ischemia period, the bolt was opened to atmospheric pressure, allowing intracranial pressure to fall rapidly. The bolt was sealed with bone wax, and a period of either 30 min or 2 hr of reperfusion followed. We have used this method of brain ischemia previously and have shown (with microspheres) that there is no blood flow to any brain region during the period of ischemia (5). Control animals were ventilated for 1–3 hr prior to measurement of tracer transport.

Determination of Residual Brain Blood Volume. Brain blood volume was determined using radioiodinated serum albumin (RISA) at: control (n = 14), 30 min after ischemia (n = 3), and 2 hr after ischemia (n = 3). Briefly, 50 μCi (iv) of RISA was allowed to circulate for 1 min, at which time a blood sample was taken, and the animals were killed with KCl. The head was then elevated to 10 cm above the heart and the brain was quickly removed without perfusion. Brain tissue was weighed and counted with the blood sample and residual brain blood volume calculated as milliliters per gram of tissue. Results were used in subsequent calculations of tracer movement into the brain.

Blood to Brain Transfer of [^{14}C]Urea, ^{22}Na, and [^{14}C]Sucrose. Catheters were placed in both femoral arteries and both femoral veins. One set of catheters was used to monitor blood pressure, sample arterial blood, and provide venous access as necessary. The other set of femoral catheters was connected together with a 5-cm length of silicon tubing to form an arteriovenous shunt. This shunt allowed for rapid, multiple sampling of arterial blood. Just prior to shunt sampling, heparin (50 units/kg) was given intravenously to maintain shunt patency.

The movement of all of the radiolabeled tracers into brain tissue was determined during control, 30 min after ischemia, and 2 hr after ischemia. Before injection, the sucrose was purified by thin layer chromatography. Our preliminary studies, as well as reports from other laboratories (6), suggest that this step is necessary. Twenty-five microcuries of tracer ([^{14}C]urea mol wt = 60.1 or ^{22}Na, New England Nuclear, Boston, MA; [^{14}C]sucrose mol wt = 342, American Radiolabeled Chemicals, Inc., St. Louis, MO) were injected as an intravenous bolus. Blood samples were taken from the arteriovenous shunt at 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 90, 180, 240, 360, 480, 600, 900, and 1,200 sec. Then the piglet was killed with KCl, the head was elevated 10 cm above the heart, and the brain was removed rapidly (within 1 min). Major vessels were dissected from the tissue and brain regions, separated, and weighed. Brain tissue was then digested and homogenized in 1 N NaOH and 0.1% sodium dodecyl sulfate. Tissue and scintillation fluid (8 ml) were then added to glass vials. Blood samples were centrifuged (20,000g for 5 min) and 50 μl of plasma were added to scintillation fluid. Corrections for quench were made from quenched standards and data were handled as disintegrations per minute per milliliter of blood or gram of tissue. Calculation of the K_{in} transfer constant ($cm^3 \cdot g^{-1} \cdot s^{-1} \times 10^6$) into the brain used the following equation, described previously by Ohno *et al.* (7):

$$K_{in} = \frac{C_i\,(T) - C_b}{\int_0^T C_p\,dt}$$

where C_i (T) = tracer concentration in brain tissues (dpm/g) at the end of the experiment; C_b = tracer concentration in the residual blood (dpm/g) at the end of the experiment; C_p = tracer concentration in plasma (dpm/g); and T = time of uptake when brain tracer content is determined.

Statistics. Values are presented as mean ± SE. Comparisons among groups were made using analysis of variance with a Scheffe post hoc test. A significance level of $P < 0.05$ was used.

Results

Mean arterial blood pressure, arterial blood gases, and pH are listed in Table I. There were no significant differences in any of these parameters during the measurement periods described in this report, and all were within the normal range for newborn pigs.

Table II lists the regional cerebral blood volume values used to correct for residual intravascular tracer. Regional cerebral blood volume was not different under any of the experimental conditions studied. We therefore used the pooled data from all of the animals studied.

[^{14}C]Urea and ^{22}Na content of brain tissue are shown in Figures 1 and 2. There was a significant rise in brain [^{14}C]urea and ^{22}Na content at 30 min after ischemia and 2 hr after ischemia. Sucrose transport shown in Figure 3 was not increased at 30 min, but by 2 hr the K_{in} for sucrose increased by more than 3-fold.

Discussion

The present experiments on newborn pigs indicate that cerebral ischemia alters the transport properties of the newborn blood-brain barrier following reperfusion.

Table I. Mean Arterial Blood Pressure, Arterial Blood Gases, and pH

	After ischemia		
	Control	30 Min	2 hr
	Urea piglets[a]		
Blood pressure (mm Hg)	62 ± 7	63 ± 8	64 ± 9
pO_2 (mm Hg)	106 ± 12	100 ± 14	98 ± 7
pCO_2 (mm Hg)	40 ± 4	42 ± 6	38 ± 3
pH	7.46 ± 0.05	7.42 ± 0.03	7.45 ± 0.03
	Sodium piglets[b]		
Blood pressure (mm Hg)	60 ± 8	58 ± 6	63 ± 8
pO_2 (mm Hg)	102 ± 8	106 ± 8	110 ± 6
pCO_2 (mm Hg)	38 ± 5	40 ± 7	42 ± 8
pH	7.45 ± 0.06	7.46 ± 0.07	7.43 ± 0.05
	Sucrose piglets[c]		
Blood pressure (mm Hg)	64 ± 6	64 ± 8	62 ± 7
pO_2 (mm Hg)	98 ± 11	102 ± 12	101 ± 7
pCO_2 (mm Hg)	42 ± 5	40 ± 6	39 ± 7
pH	7.43 ± 0.04	7.45 ± 0.02	7.44 ± 0.04

[a] Control, $n = 8$; 30 min, $n = 8$; 2 hr, $n = 7$.
[b] Control, $n = 11$; 30 min, $n = 7$; 2 hr, $n = 6$.
[c] Control, $n = 5$; 30 min, $n = 5$; 2 hr, $n = 5$.

Table II. Regional Cerebral Blood Volumes under Control, at 30 Min of Reperfusion, and at 2 Hr of Reperfusion[a]

	Cerebrum	Caudate	Midbrain	Pons	Medulla	Cerebellum
Control (no ischemia, $n = 9$)	0.012 ± 0.001	0.012 ± 0.005	0.012 ± 0.003	0.015 ± 0.003	0.013 ± 0.004	0.015 ± 0.004
30 min ($n = 5$)	0.011 ± 0.001	0.012 ± 0.003	0.010 ± 0.003	0.015 ± 0.004	0.014 ± 0.003	0.014 ± 0.003
2 hr ($n = 6$)	0.013 ± 0.002	0.012 ± 0.006	0.013 ± 0.005	0.014 ± 0.005	0.013 ± 0.005	0.015 ± 0.003
Pooled[a]	0.011 ± 0.001	0.011 ± 0.001	0.011 ± 0.001	0.015 ± 0.002	0.013 ± 0.002	0.015 ± 0.002

[a] Regional cerebral blood volumes calculated as milliliters per gram of tissue.
[b] Pooled data are the mean ± SE of all animals studied.

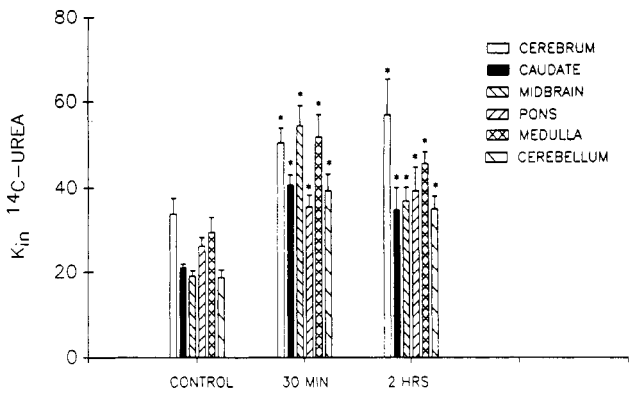

Figure 1. K_{in}: transfer constant for [^{14}C]urea ($cm^3 \cdot g^{-1} \cdot s^{-1} \times 10^6$) at the three experimental conditions studied: control ($n = 8$), 30 min after ischemia ($n = 8$), and 2 hr after ischemia ($n = 7$). Values are mean ± SE. * $P < 0.05$ versus control.

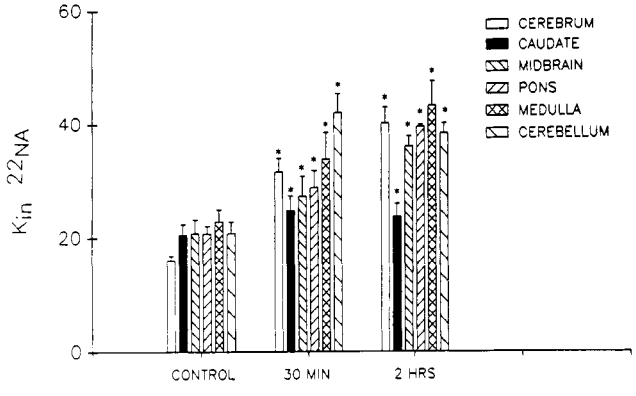

Figure 2. K_{in}: transfer constant for ^{22}Na ($cm^3 \cdot g^{-1} \cdot s^{-1} \times 10^6$) at the three experimental conditions studied: control ($n = 11$), 30 min after ischemia ($n = 7$), and 2 hr after ischemia ($n = 6$). Values are mean ± SE. * $P < 0.05$ versus control.

Alterations are observed at 30 min and persist at 2 hr following ischemia. However, in contrast to other tracers examined, sucrose transport was not increased at 30 min of reperfusion, but was increased 2 hr after injury, suggesting that factors other than molecular size (sucrose, mol wt 342; urea, mol wt 60.1; sodium, mol wt 23) and lipid solubility affect blood-brain barrier transport after ischemia. Several mechanisms which can be molecule specific could account for the increased blood-brain barrier transport observed in these studies, including transport through endothelial cell tight junctions, diffusion through the cells, or other transport

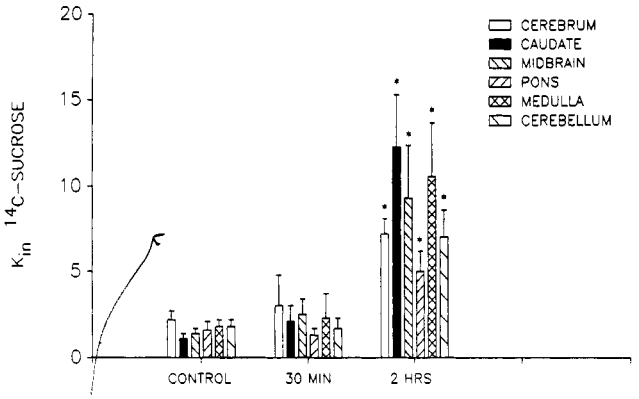

Figure 3. K_{in}: transfer constant for [^{14}C]sucrose ($cm^3 \cdot g^{-1} \cdot s^{-1} \times 10^6$) at the three experimental conditions studied: control ($n = 5$), 30 min after ischemia ($n = 5$), and 2 hr after ischemia ($n = 5$). Values are mean ± SE. * $P < 0.05$ versus control.

mechanisms.

The pattern of responses observed may have implications regarding the mechanism of entry of the tracers into the brain. Transport into the brain of substances with low lipid solubility indicates passage between cells or nondiffusional transport through cells. Opening passageways between cells or water-filled channels through cells would permit movement that is largely dependent upon molecular weight and charge. However, transport of urea and sodium into the brain following ischemia occurs at a time when sucrose entry has not increased, suggesting that these three tracers are not moving across the blood-brain barrier via the same mechanism. Alterations in specific transfer of other substances may be occurring as well. Interestingly, in preliminary studies we have found that chloralose anesthesia can induce sucrose transport but not transport of other tracers, suggesting that there could be specific transport mechanisms for each molecule.

An increase in cerebral blood flow, with a further recruitment of perfused capillaries, would cause an apparent increase in permeability. However, we know from previous experiments (4) that blood flow to all areas of the piglet brain except the cerebrum is not different from pre-ischemia levels at 15, 20, 40, 90 min, and 24 hr after ischemia. Blood flow to the cerebrum is normal at 15 and 20 min, reduced at 40 and 90 min, and has returned to control at 24 hr of reperfusion. Therefore, the increased blood to brain transfer that we observed was not caused by increases in blood flow since no increase in blood flow occurs at any reperfusion time examined.

Following ischemia-reperfusion, increased transport of sucrose, urea, and sodium was observed. Dobbins *et al.* (8) found increased movement of ^{14}C-aminoisobutyric acid into the brain of adult rats 15 min, 3 hr, and 6 hr following forebrain ischemia with a recovery of the barrier to ^{14}C-aminoisobutyric acid after 24 hr of reperfusion. However, they did not detect movement of albumin into the brain using Evans blue dye. The authors concluded that the postischemic opening of the blood-brain barrier was confined to smaller molecules. Our results indicate that the chronology of barrier alterations may depend upon the substance in question. Both the present study and the previous report support the concept that ischemia-reperfusion injury alters blood-brain barrier properties.

^{125}I-Albumin was used to determine the residual blood volume for the sucrose experiments. The quantity of blood left in the brain vascular system is of critical importance to the determination of tracer transport. Because the determination of residual blood volume can introduce significant error into the calculation of tracer movement into the brain (9), we studied the RISA space in a number of animals without ischemia and at each of the time points following ischemia. There were no significant or apparent differences in the residual blood volume under these varying conditions (Table II). Therefore, we used the pooled data from these studies to increase the accuracy of the measurement. Furthermore, although studied in different species, other authors (10) have reported similar values for residual brain blood volume. With albumin it is also possible that the endothelial cell luminal membrane is altered, resulting in movement into or binding of RISA by the endothelial cell. We cannot completely discount this possibility, but it seems less likely considering that the brain-blood volume measured using RISA was not increased at 30 min and 2 hr after ischemia, and that the remaining tracers showed a change in transport.

The single-time point method used to study sucrose, urea, and sodium transport assumes that the rate of tracer entry into the brain is approximately proportional to its concentration in plasma and that the amount of tracer lost from brain by reflux back into the blood is negligible (11). Backflux will be negligible when the time of the experiment is chosen appropriately. In the present study, we used a 20-min circulation time, as 20 min meets previously published time limits defined by Smith (9), including a less than 10% tracer backflux and a small (<33%) contribution by residual intravascular tracer.

Recent evidence suggests that the blood-brain barrier of several newborn species, including man, is anatomically closed at birth (1). Additional evidence from newborn rabbit studies suggests that the newborn blood-brain barrier is closed and transports substances selectively at birth. This selectivity includes systems for uptake of choline, adenine, and arginine (2), which would be necessary substrates for a developing brain. Such differences in the properties of the newborn blood-brain barrier compared to the adult make a different response to ischemia-reperfusion injury possible during the newborn period, and further suggest that the movement across the neonatal blood-brain barrier must be

considered differently from the adult species.

These experiments show postischemic alterations in the blood-brain barrier of newborn pigs. The pattern of increased blood to brain transfer of the tracers studied suggests that molecular weight cannot totally account for transport changes.

This study was supported by grants from the National Institutes of Health and a Clinical Investigator Award from NIH (R. M.).

1. Johanson CE. Ontogeny and phylogeny of the blood-brain barrier. In: Neuwelt EA, Ed. Implications of the Blood-Brain Barrier and Its Manipulation: Basic Science Aspects. New York: Plenum Medical Book, Vol **1**: p158, 1989.
2. Braun LD, Cornford EM, Oldendorf WW. Newborn rabbit blood-brain barrier is selectively permeable and differs substantially from the adult. J Neurochem **34**:147–152, 1980.
3. Sundt TM Jr, Waltz AG. Cerebral ischemia and reactive hyperemia. Studies of cortical blood flow and microcirculation before, during, and after temporary occlusion of middle cerebral artery of squirrel monkeys. Circ Res **28**:426–433, 1971.
4. Leffler CW, Busija DW, Mirro R, Armstead WM, Beasley DG. Effects of ischemia on brain blood flow and oxygen consumption of newborn pigs. Am J Physiol **257**:H1917–H1926, 1989.
5. Leffler CW, Busija DW, Beasley DG, Armstead WM, Mirro R. Postischemic cerebral microvascular responses to norepinephrine and hypotension in newborn pigs. Stroke **20**:541–546, 1989.
6. Preston E, Haas N. Defining the lower limits of blood-brain barrier permeability: Factors affecting the magnitude and interpretation of permeability-area product. J Neurosci Res **16**:709–719, 1986.
7. Ohno K, Pettigrew KD, Rapoport SI. Lower limits of cerebrovascular permeability to non-electrolytes in the conscious rat. Am J Physiol **235**:H299–H307, 1978.
8. Dobbins J, Crockarol HA, Ross-Russell R. Transient blood-brain barrier permeability following profound temporary global ischemia. An experimental study using ^{14}C-AIB. J Cereb Blood Flow Metab **9**:71–78, 1989.
9. Smith QR. Quantitation of blood-brain barrier permeability. In: Neuwelt EA, Ed. Implications of the Blood-Brain Barrier and Its Manipulation: Basic Science Aspects. New York: Plenum Medical Book, Vol **1**: pp102–103, 1989.
10. Gross PM, Teasdale GW, Graham DI, Angerson WJ, Harper AM. Intraarterial histamine increases blood-brain transport in rats. Am J Physiol **243**:H307–H317, 1982.
11. Blasberg RG, Patlak CS, Fenstermacher JD. Selection of experimental conditions for the accurate determination of blood-brain transfer constants from single-time experiments: A theoretical analysis. J Cereb Blood Flow Metab **3**:215–225, 1983.