Regional Cerebral Blood Flow During Hypoxia-Ischemia in Immature Rats

Robert C. Vannucci, MD, David T. Lyons, MD, and Francesca Vasta, BS

Immature rats subjected to a combination of unilateral common carotid artery ligation and hypoxia sustain brain damage confined largely to the ipsilateral cerebral hemisphere. To ascertain the extent and distribution of ischemic alterations in the brains of these small animals, we modified the Sakurada technique to measure regional cerebral blood flow using carbon-14 autoradiography. Seven-day-old rats underwent right common carotid artery ligation following which they were rendered hypoxic with 8% O₂ at 37°C. Before and during hypoxia, the rat pups received an injection of iodol-[14C]antipyrine for determination of regional cerebral blood flow. Blood flows to individual structures of the ipsilateral cerebral hemisphere were not influenced by arterial occlusion alone; flows to the contralateral hemisphere and to the brainstem and cerebellum actually increased by 25–50%. Hypoxia-ischemia was associated with decreases in regional cerebral blood flow of the ipsilateral hemisphere such that by 2 hours, flows to subcortical white matter, neocortex, striatum, and thalamus were 15%, 17%, 34%, and 41% of control, respectively. The hierarchy of the blood flow reductions correlated closely with the distribution and extent of ischemic neuronal necrosis. However, unlike the pathologic pattern of this model, the degree of ischemia appeared homogeneous within each brain region. Blood flows to contralateral cerebral hemispheric structures were relatively unchanged from prehypoxic values, whereas flows to the brainstem and cerebellum nearly doubled and tripled, respectively. Thus, ischemia is the predominant factor that determines the topography of tissue injury to major regions of immature rat brain, whereas metabolic factors (intrinsic vulnerability) may influence the heterogeneous pattern of damage seen within individual structures. (Stroke 1988;19:245–250)

A critical question in perinatal medicine is whether hypoxia alone is capable of damaging the brain of a fetus or newborn infant or whether cerebral ischemia, with or without concurrent systemic hypoxia, is necessary for tissue injury to occur. Investigations in animals suggest that hypoxia alone does not damage the immature brain and that superimposed ischemia, produced either by arterial occlusion (stroke) or by systemic hypotension, is a necessary prerequisite for tissue destruction. If this observation can be extended to human infants, then hypoxemia alone should be relatively innocuous so long as the cerebral arteries remain patent and systemic hypotension is avoided.

We recently developed an experimental model of perinatal hypoxic-ischemic brain damage in developing rats. The technique involves the ligation of one common carotid artery followed by exposure of the rat to systemic hypoxia for 2 or more hours. We assumed that the single carotid artery occlusion provided the ischemia necessary to damage the immature brain, although cerebral perfusion was not assessed directly in the original investigation. In our present study, we measured regional cerebral blood flow (rCBF) using iodol-[14C]antipyrine to confirm the presence of cerebral ischemia and to determine its extent and distribution. The findings are discussed in relation to the pattern and severity of brain damage seen in our immature rat model.

Materials and Methods

Dated, pregnant Sprague-Dawley rats were purchased from a commercial breeder, housed individually, and fed standard laboratory chow ad libitum. Offspring, delivered vaginally, were reared with their dams for 7 days. Individual rat pups were anesthetized with 4% halothane induction, 1% halothane maintenance in 30% oxygen and the balance nitrous oxide. The neck was incised in the longitudinal plane for 5 mm; the right common carotid artery was identified, ligated with 3-0 surgical silk. The neck wound then was sutured, and the rat was returned to its dam for 4 hours. The rats then were placed in air-tight 500-ml jars partially submerged in a 37°C water bath to maintain a constant thermal environment. The jars were flushed with a warmed and humidified gas mixture of 8% oxygen: 92% nitrogen via inlet and outlet portals. The rat pups were exposed to hypoxia for 60 or 120 minutes, at which times rCBF was determined (see below). Ligation rats were littermates undergoing carotid artery ligation without hypoxia, and control rats were subjected to neither carotid artery ligation nor hypoxia. rCBF was determined in the immature rats by the iodol-[14C]antipyrine fractional extraction technique as originally described by Sakurada et al and as modified in our laboratory for very small animals. Either before or during hypoxia, each rat pup received a s.c. injection.
of 5 μCi 4-iodo-(N-methyl-14C)-antipyrine (New England Nuclear, Boston, Massachusetts) in 0.1 ml normal saline. The injection was made into the back roughly in the midline, and the rat was discarded if any bleeding or leakage from the injection site occurred. At either 0.0, 0.25, 0.5, 1.0, 1.5, or 2.0 minutes after the injection the rat pup was decapitated, and arterialized blood was collected from the severed neck vessels into a heparinized glass capillary tube. Ten microliters of blood was pipetted from the capillary tube into a scintillation vial containing 1 ml Soluene-350 (United Technologies Packard, Downers Grove, Illinois). After mixing overnight in a mechanical shaker, the solution was combined with 9 ml Demilume-36 (United Technologies Packard). The samples then were isotopically counted in a liquid scintillation spectrometer.

The brains of the rat pups killed 2 minutes after injection of iodo[14C]antipyrine were rapidly removed from the skulls and immediately frozen whole in liquid Freon at −70° C. Coronal sections of the brain were cut to a thickness of 20 μm in a cryostat (American Optical, Buffalo, New York) kept at −12° C. The brain sections then were mounted on glass slides, dried at 55° C on a hot plate, and subjected to quantitative carbon-14 autoradiography along with [14C]methyl methacrylate standards (Amersham, Arlington Heights, Illinois). A comparison of the optical densities of portions of the autoradiograms corresponding to specific brain regions with those of the carbon-14 standards yielded concentrations (in disintegrations per minute per gram) of iodo[14C]antipyrine.

rCBF for specific regions of rat brain was calculated according to an equation derived from the Fick equation by Kety:

$$C_a(T) = \lambda \times K \int_0^T C_s \times e^{-\lambda(T-t)} dt$$

where $C_a(T)$ = concentration of tracer in the brain at time $T$ (dpm/g), $\lambda$ = brain: blood partition coefficient (ml/g) = 0.94, $C_s$ = concentration of tracer in the blood (dpm/ml), $T$ = time at end of the experiment (2 minutes), and $t$ = some time between 0 and $T$. To solve the equation, a series of substitutions were made for $K$. The value of the integral was estimated using the trapezoidal rule. That $K$ providing the calculated value of $C_a(T)$ closest to the observed value provided the solution. This $K$ multiplied by $\lambda$ equals blood flow in milliliters per gram per minute.

We are aware of certain limitations in the application of the indicator diffusion rCBF technique to small, immature rats. A major modification of the method included the use of several littersmates rather than a single rat to ascertain the blood saturation curve of the isotope. Validation studies indicate that the use of many animals and the associated random variation of the counts derived from the timed blood samples minimally influences the character of the blood saturation curve and the resultant blood flow calculated. Differences in rCBF for any single animal would not be subject to random error since all regional data are derived from the same blood saturation curve.

An additional criticism of the use of our technique in small animals relates to the necessity of obtaining "arterialized" blood from severed neck vessels rather than directly from an artery. It simply is not feasible to catheterize a large blood vessel in small animals without severely compromising systemic physiologic status. We have found that blood obtained from the severed trunk has an oxygen saturation of 85%, indicating that the blood is essentially of arterial origin. Assuming that venous blood contains no radioisotope (highly unlikely), a 10% reduction in the blood saturation curve would overestimate rCBF values by only 6–9%. Given the high oxygen saturations observed, we concluded that any error produced by the blood collection method must be small.

Analysis of variance and the two-tailed Student's $t$ test were used to analyze the data.

**Results**

The data shown in Table 1 depict rCBF values for nine component structures of the immature rat brain. In control rats, blood flow to homologous regions of the cerebral hemispheres, brainstem, and cerebellum were near identical (Figures 1 and 2). As in perinatal animals of other species, blood flow to the brainstem was higher than that of the cerebral cortex and diencephalic gray matter nuclei, while subcortical white matter exhibited the lowest flow value.

Common carotid artery ligation alone was associated with increased blood flow to all cerebral hemispheric structures contralateral to the arterial occlusion, although most of the values did not reach significance (Table 1). The brainstem and cerebellum participated in this mild hyperemia, despite the fact that these two structures are perfused predominantly or exclusively by the vertebrobasilar system of arteries. Blood flow to regions ipsilateral to the carotid artery occlusion remained relatively unchanged from control values. The data indicate quite adequate collateral circulation to the ipsilateral cerebral hemisphere, a finding that accounts for the absence of neuropathology in immature rats subjected to unilateral common carotid artery occlusion without superimposed hypoxia.

Unilateral common carotid artery ligation combined with systemic hypoxia led to profound changes in rCBF at 1 and 2 hours (Table 1, Figures 3–5). In all cerebral hemispheric structures analyzed save one (the hypothalamus), blood flow ipsilateral to the arterial occlusion was reduced by 60% or more, indicating the presence of ischemia in these regions. Furthermore, the ischemia was progressive, at least in the cerebral cortex, since blood flows consistently were lower at 2 hours than at 1 hour. The most severely affected structures included the frontal and parietal cerebral cortex and subcortical white matter; however, the blood flow reductions to other component structures were of such a degree and duration that ultimate ischemic neuronal necrosis or infarction would have been expected in all regions.

Blood flow to individual structures of the contralateral cerebral hemisphere remained stable during
TABLE 1. Regional Cerebral Blood Flow During Hypoxia-Ischemia Induced by Common Carotid Artery Ligation in Immature Rats

<table>
<thead>
<tr>
<th>Structure</th>
<th>Control (n = 8)</th>
<th>Ligation (n = 6)</th>
<th>1 hour (n = 12)</th>
<th>2 hours (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>42 ± 6</td>
<td>62 ± 9*</td>
<td>48 ± 7</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>40 ± 5</td>
<td>42 ± 7</td>
<td>16 ± 3†</td>
<td>8 ± 17†</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>42 ± 7</td>
<td>57 ± 11</td>
<td>48 ± 8</td>
<td>53 ± 12</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>39 ± 6</td>
<td>38 ± 7</td>
<td>12 ± 2†</td>
<td>6 ± 17†</td>
</tr>
<tr>
<td>Thalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>30 ± 2</td>
<td>41 ± 9</td>
<td>44 ± 6</td>
<td>39 ± 3</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>29 ± 3</td>
<td>34 ± 6</td>
<td>16 ± 2∗</td>
<td>12 ± 2†</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>36 ± 4</td>
<td>54 ± 6</td>
<td>54 ± 8</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>35 ± 4</td>
<td>43 ± 8</td>
<td>26 ± 4</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>30 ± 4</td>
<td>46 ± 5</td>
<td>36 ± 4</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>29 ± 3</td>
<td>37 ± 5</td>
<td>12 ± 2†</td>
<td>9 ± 3†</td>
</tr>
<tr>
<td>Putamen/globus pallidus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>26 ± 2</td>
<td>38 ± 8</td>
<td>40 ± 6</td>
<td>25 ± 9</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>26 ± 2</td>
<td>30 ± 6</td>
<td>12 ± 2†</td>
<td>10 ± 2†</td>
</tr>
<tr>
<td>Subcortical white matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>18 ± 2</td>
<td>25 ± 5</td>
<td>23 ± 3</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>18 ± 2</td>
<td>18 ± 3</td>
<td>5 ± 1†</td>
<td>3 ± 1†</td>
</tr>
<tr>
<td>Brainstem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>50 ± 9</td>
<td>63 ± 12</td>
<td>72 ± 10</td>
<td>90 ± 18*</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>49 ± 7</td>
<td>61 ± 11</td>
<td>70 ± 11</td>
<td>71 ± 8</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contralateral</td>
<td>29 ± 3</td>
<td>42 ± 8</td>
<td>49 ± 7*</td>
<td>87 ± 19*</td>
</tr>
<tr>
<td>Ipsilateral</td>
<td>29 ± 4</td>
<td>41 ± 9</td>
<td>46 ± 6*</td>
<td>68 ± 11*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.  
* p<0.05, † p<0.001 compared with control values; ‡ p<0.05 compared with values at 1 hour.

Discussion

The model of perinatal hypoxic-ischemic brain damage described here was the first to be developed in small laboratory animals and was based on the Levine preparation in adult rats, that is, unilateral common carotid artery ligation combined with hypoxia. Levine was unable to produce brain damage in adult rats by anoxia (nitrogen exposure) alone, whereas the addition of unilateral common carotid artery ligation resulted in ischemic neuronal alterations of the ipsilateral cerebral hemisphere in a large proportion of rats. As in adults, the brains of immature rats cannot be damaged by hypoxia or anoxia alone; thus, the Levine preparation was required to produce unequivocal brain damage in the majority of rats. It was assumed, although not confirmed, that the addition of unilateral common carotid artery ligation to systemic hypoxia provided the prerequisite element of cerebral ischemia required for tissue injury to occur. The present investigation documents that cerebral ischemia indeed does exist in this model of perinatal brain damage.

It must be emphasized that in the adult rat Levine preparation, unilateral common carotid artery ligation produces ischemia that is relative rather than absolute. In adult rats subjected to hypoxia with arterial oxygen tensions of 20 mm Hg, ipsilateral cortical blood flow, measured with [14C]ethanol, actually increases albeit to not as great a degree as in the contralateral cerebral hemisphere. Similar findings have been reported by Ginsberg et al, who measured rCBF with [14C]antipyrine in several regions of the adult rat brain and found flows that ranged from 95 to 156% and 143 to 200% of control in the ipsilateral and contralateral cerebral hemispheres, respectively. In both investigations, the rats were lightly anesthetized, paralyzed, and artificially ventilated; these procedures were used to minimize cardiorespiratory compromise during hypoxemia with its accompanying systemic hypotension. In this important aspect, these studies differ from ours in which the immature rat pups were unanesthetized and spontaneously breathing during hypoxia, during which time a modest decline in systemic blood pressure (−25%) occurred (see below).

In contrast to the adult Levine preparation, the focal cerebral ischemia resulting from unilateral common carotid artery ligation and hypoxia in immature rats was absolute rather than relative. Although not total, the ischemia was severe enough to reduce regional blood flows to the ipsilateral cerebral hemisphere to below values known to produce neurologic deficits and/or brain damage in adult animals subjected to focal or global cerebral ischemia without concurrent hypoxia. Furthermore, a previous investigation from our laboratory has shown that hypoxia-ischemia in rat pups leads rapidly to a near-complete exhaustion of glucose in the ipsilateral hemisphere, indicating that glucose, as well as oxygen, availability to the brain is curtailed by ischemia. The limited glucose delivery, in turn, retards anaerobic glycolytic flux required to maintain cellular ATP and phosphocreatinine under hypoxic conditions. As a result, these energy reserves are depleted and ultimate tissue damage occurs.

Our model of hypoxia-ischemia was associated with decreases in rCBF to individual structures of the ipsilateral cerebral hemisphere, such that by 2 hours flows to the neocortex, striatum (caudate nucleus and putamen/globus pallidus), and thalamus were 17, 34, and 41% of control, respectively. This hierarchy of blood flow reduction correlates closely with the dis-
FIGURE 1. Autoradiogram of coronal section of brain at level of the hippocampus in 7-day-old control rat (no ligation, no hypoxia). Darker regions correspond to areas of greater blood flow. Easily discernible structures include parietal cerebral cortex (CC), subcortical white matter (WM), hippocampus (HC), thalamus (TM), and hypothalamus (HT).

FIGURE 2. Autoradiogram of coronal section of brain at level of the brainstem and cerebellum in 7-day-old control rat. Structures include cerebellar gray (GM) and white (WM) matter, inferior colliculus (IC), and brainstem (BS).

tribution and extent of ischemic neuronal necrosis and/or infarction previously reported from our laboratory. Thus, neuropathologic changes were most frequent and most severe in the posterior cerebral cortex, where 92% of 25 rats exhibited some degree of damage and fully 56% showed evidence of infarction. Striatum and thalamus were damaged in 84 and 68% of the rats, respectively, with corresponding lesser frequencies of infarction. Unfortunately, we were unable to assess the extent of ischemia in the hippocampus, owing to the fact that microdensitometric readings (2 mm diameter) would have included some surrounding white matter. However, visual inspection of the autoradiograms indicated that the hippocampus was affected to a degree similar to or worse than that of the cerebral cortex (Figures 3 and 4), in keeping with the 88% incidence of morphologic damage and 48% incidence of infarction in this structure. Blood flow to the subcortical white matter was reduced to the greatest extent of all the brain regions examined (15% of control); injury to this structure always coexisted with gray matter damage and occasionally was seen in...
FIGURE 3. Autoradiogram at level of the hippocampus in 7-day-old rat subjected to unilateral common carotid artery ligation and 1 hour of hypoxia with 8% oxygen. Note reduced blood flow (lighter areas) to all component structures of ipsilateral (right side) cerebral hemisphere.

isolation (R.C. Vannucci and J.B. Brierley, unpublished data). This finding emphasizes the vulnerability of immature white matter to hypoxia-ischemia. Blood flows to individual structures of the contralateral cerebral hemisphere were relatively unchanged from prehypoxic values, which of itself is of interest since systemic hypoxia alone is associated with one- to twofold increases in rCBF in perinatal animals of other species. The most likely explanation for the blunted response to hypoxia was the presence of systemic hypotension secondary to the hypoxic cardiovascular depression known to occur in these animals. Such hypotension would abolish any hyperemic response to hypoxia in the presence of a dilated cerebrovascular bed, that is, loss of autoregulation. Additionally, hypocapnia secondary to hyperventilation may have contributed to the blunted response of the contralateral cerebral hemisphere to hypoxia. Blood flows to the brainstem and cerebellum increased, indicating a preferential perfusion of those brain regions vital to respiratory and cardiovascular stability. Hyperemia of hindbrain structures previously has been demonstrated during hypoxia with or without superimposed systemic hypotension and/or hypocapnia in fetal sheep and monkeys and in newborn dogs and piglets.

Although the extent of ischemia among several gray matter regions of the ipsilateral cerebral hemisphere correlated closely with the distribution and extent of ischemic tissue necrosis, no such correspondence existed within individual brain structures. Specifically, the degree of ischemia always appeared homogeneous within each brain structure, whereas ischemic neuronal destruction followed distinctive patterns. In the neocortex, tissue injury showed either a laminar emphasis (layers 3 and 5 + 6) or a contrasting pattern of alternating columns of normal and damaged neurons oriented at right angles to the pial surface. In the hippocampus, ischemic damage was frequent in the Sommer sectors hi and h3–5, with relative preservation of h2. In the striatum, damage was maximal medially...
toward the ependyma of the lateral ventricle; in the thalamus, damage was always focal and usually located in the lateral half. In this regard, we have shown columnar alterations in NADH fluorescence (as a reflection of cellular oxidation-reduction state) in the same location of neocortex and hippocampus as histologically verified tissue damage. Thus, the degree and duration of ischemia are the predominant factors that determine the topography of injury to major regions of the immature rat brain, whereas metabolic factors (intrinsic vulnerability) primarily influence the heterogeneous pattern of damage seen within individual structures.

References
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