Beagle puppy model of perinatal cerebral insults

Cerebral blood flow changes and intraventricular hemorrhage evoked by hypoxemia

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Asphyxia, with its attendant hypoxemia, is by far the most common cause of neonatal cerebral infarction, and frequently results in lesions of the parieto-occipital white matter in addition to other neuropathological changes. This study examines the effects of hypoxemia on regional cerebral blood flow (CBF) in the newborn beagle pup. The animals were anesthetized, underwent a tracheotomy, and were paralyzed. Pups were randomly divided into two groups: one group was subjected to hypoxemia produced by altering the oxygen concentration in the inspired air, and the other received no insult. In the hypoxic pups, the pO2 was 13.1 ± 2.1 mm Hg (mean ± standard deviation). Autoradiographic determinations of CBF were performed by the carbon-14-iodoantipyrine technique 15 minutes after randomization. Significant increases in CBF were found throughout the brains of the hypoxic pups. The CBF was increased to cortical and central gray regions and to frontal and temporal white matter but was unchanged in the parietal white matter, one of the classic sites of radiological and neuropathological injury in neonates with perinatal asphyxia. An unexpected finding was the increased incidence of germinal matrix and/or intraventricular hemorrhages in the hypoxic pups.

Key Words: microvasculature • prostaglandin • cerebral blood flow • asphyxia • hypoxemia • beagle pup

Although the development of sophisticated perinatal intensive care has brought steady improvement in the mortality rate among small and critically ill neonates, the incidence of major neurodevelopmental abnormalities has remained essentially unchanged in this patient population over the past decade. Increasing attention, therefore, has been paid to the early identification of those infants at risk for neurodevelopmental handicaps. Recent data have demonstrated that neonates with parenchymal involvement of intraventricular hemorrhage (IVH) and those with perinatal cerebral infarction may constitute two such groups. Intraventricular hemorrhage or hemorrhage into the germinal matrix tissues (GMH) with possible rupture into the ventricular system and parenchymal involvement of the developing cerebrum remains a common problem in preterm neonates. In addition, IVH originating in the choroid plexus may occur in both pre- and full-term infants. Severe asphyxia has been reported to occur in 0.2% to 2% of live births, and perinatal cerebral infarction (or stroke) is a well-known manifestation of this disorder. Studies of neonatal animals and newborn infants have demonstrated that both IVH and perinatal infarction may be associated with alterations in regional cerebral blood flow (CBF) and have led to the hypothesis that the neuropathological and clinical sequelae associated with each insult may be secondary to such.

The newborn beagle pup provides a good model for the study of the developing brain. Materials and Methods

Newborn beagle pups (24 to 96 hours old) were anesthetized with intraperitoneal pentobarbital, then
subjected to tracheotomy under local anesthesia (1% xylocaine); they were then paralyzed with pancuronium bromide (1 mg/kg subcutaneously) and artificially ventilated with a mixture of 30% oxygen and 70% nitrous oxide (for analgesia). Under local anesthesia, bilateral femoral venous and arterial lines were inserted and arterial blood pressure was monitored utilizing a pressure transducer and polygraph recorder. Body temperature was recorded by a thermal probe and maintained by means of a warming light at 36.5° to 37.5°C. Ventilatory rate and tidal volume were adjusted to maintain arterial normoxia (> 40 mm Hg) and normocapnia (30 to 40 mm Hg).

When the pups were physiologically stabilized, they were randomly assigned to either a hypoxemia group or a control group. Hypoxemia was induced by altering the oxygen concentration in the inspired air until pO2 values reached 10 to 20 mm Hg. Control pups were not made hypoxic but were observed for a comparable period of time. Fifteen minutes following randomization to insult or no insult and the accomplishment of the pO2 values for the hypoxic pups, all animals underwent carbon-14-labeled iodoantipyrine (14C-IAP) measurements of CBF and were rapidly sacrificed thereafter.

Cerebral Blood Flow Studies

The CBF determinations were made by a bolus venous infusion of 50 μCi 14C-IAP simultaneously with the rapid arterial withdrawal of blood into an artificial organ system composed of approximately 80 cm of polyethylene tubing (PE-60) attached to a Harvard infusion/withdrawal pump* calibrated to withdraw blood at a constant rate of 2.72 ml/min. At the end of this 5-second interval, the animals were rapidly decapitated and the brains were removed and placed in isopentane chilled to ~60°C. Brain sections 32 μ thick were prepared with a cryostat maintained between ~15° and ~10°C, and every 25th section was placed on a glass slide, dried on a hot plate at 60° to 70°C, and placed sequentially for 7 days in an x-ray cassette loaded with Kodak SB-5 film. Calibrated plastic standards† were placed adjacent to the tissue sections. Local tissue concentrations were determined by densitometric measurements.‡

Arterial blood withdrawn during the 14C-IAP venous injection was placed in preweighed scintillation vials, and aliquots removed in triplicate were treated with an equal volume of hydrogen peroxide. Determinations of 14C were made with a Packard scintillation counter.§ using standard liquid spectrometry.

*Cerebral Blood Flow Determinations

Cerebral blood flow values were determined utilizing the following formulation:

\[
\text{CBF (ml/100 gm/min)} = \frac{(\mu Ci \text{ in brain/gm}) \times (2.22 \times 10^3 \text{ dpm/μCi})(2.72 \text{ ml/min})}{(\text{total dpm in syringe blood})}
\]

Chi-squared and unpaired t-tests were employed for statistical analysis.

Results

Physiological Data

The 14C-IAP determinations of CBF were performed on 15 pups, eight of which had been randomized to receive hypoxemia. Arterial blood gas values and animal weights are listed in Table 1; no significant differences in any of these parameters were noted prior to the initiation of hypoxemia. Following 15 minutes of hypoxemia, the pO2 value for the experimental group was 13.1 ± 2.12 mm Hg (mean ± standard deviation), compared to 87.0 ± 14.3 mm Hg for the control animals (p < 0.01). There were no significant differences in mean arterial blood pressure for the control and hypoxic pups at any time during the period of measurement.

Cerebral Blood Flow Determinations

Cerebral blood flow values are available for all seven of the control pups and, because of the technical problems, for only seven of the eight hypoxic animals (Table 2). For the control pups, the CBF values for the frontal, temporal, and parietal gray matter, respectively, were 29.7 ± 13.4, 28.3 ± 13.8, and 36.8 ± 19.5 ml/100 gm/min. The values for the same regions in the hypoxic pups were 89.3 ± 17.8, 82.7 ± 17.6, and 87.8 ± 8.9 ml/100 gm/min, respectively. Thus, for all of the gray matter regions studied, there was a significant increase in CBF in the hypoxic pups (p < 0.01 for all).

The CBF values for the frontal, temporal, and parietal white matter regions of the control pups were 5.7 ± 2.8, 6.5 ± 3.3, and 11.6 ± 7.4 ml/100 gm/min, respectively, compared to 13.4 ± 5.2, 18.9 ± 9.7, and 9.4 ± 5.1 ml/100 gm/min for the same regions in the hypoxic pups. Thus, there was a significant increase in flow to both the frontal and temporal white matter zones (p < 0.01 for both). Such an increase was not detected in the parietal white matter of hypoxic pups. Similarly, significant increases in flow to the caudate nucleus region and germinal matrix areas of the hypoxic pups were documented (Table 2).

Incidence of GMH/IVH

Six of the eight pups exposed to hypoxemia had GMH/IVH, compared to one of the seven control pups (x^2 = 5.52, p < 0.02). Of the six hypoxic pups with hemorrhage, one had GMH, two had GMH with intraventricular extension (GMH/IVH), and three had IVH of choroid-plexus origin. The single control animal with hemorrhage had GMH.
CBF and IVH in experimental hypoxemia

### TABLE 1

<table>
<thead>
<tr>
<th>Factor</th>
<th>Control Group</th>
<th>Hypoxemia Group</th>
<th>p Value</th>
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</thead>
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<tr>
<td>no. of pups</td>
<td>7</td>
<td>8</td>
<td></td>
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<tr>
<td>body weight (gm)</td>
<td>283 ± 65</td>
<td>281 ± 97</td>
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<td>0 minutes</td>
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<tr>
<td>pO2 (mm Hg)</td>
<td>88.4 ± 10.2</td>
<td>85.6 ± 11.0</td>
<td>NS</td>
</tr>
<tr>
<td>pCO2 (mm Hg)</td>
<td>34.5 ± 3.01</td>
<td>33.3 ± 2.04</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.39 ± 0.05</td>
<td>7.37 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>65.0 ± 7.8</td>
<td>62.4 ± 8.4</td>
<td>NS</td>
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<td>15 minutes</td>
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</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>87.0 ± 14.3</td>
<td>13.1 ± 2.12</td>
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</tr>
<tr>
<td>pCO2 (mm Hg)</td>
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<td>34.6 ± 2.42</td>
<td>NS</td>
</tr>
<tr>
<td>pH</td>
<td>7.36 ± 0.08</td>
<td>7.27 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>61.4 ± 8.4</td>
<td>63.8 ± 9.6</td>
<td>NS</td>
</tr>
<tr>
<td>GMH/IVH</td>
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<td></td>
</tr>
<tr>
<td>caudate nucleus</td>
<td>28.0 ± 15.4</td>
<td>66.3 ± 14.1</td>
<td>&lt;0.01</td>
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<tr>
<td>germinal matrix</td>
<td>6.28 ± 1.9</td>
<td>16.3 ± 2.1</td>
<td>&lt;0.01</td>
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</table>

* Values are means ± standard deviations. MABP = mean arterial blood pressure; NS = not significant; GMH = germinal matrix hemorrhage; IVH = intraventricular hemorrhage.

### Discussion

The study of CBF and metabolism has markedly improved the understanding of many neonatal neurological insults. Numerous studies have correlated events such as hypercarbia, volume expansion, seizures, and pneumothoraces (all of which are known to alter CBF) with IVH, and increases in CBF have been reported in infants with hypercapnia, volume expansion, and seizures, all of whom subsequently experienced GMH/IVH. In addition, studies of animal models have demonstrated that acute hypercapnia, hypertension, and hemorrhagic hypotension followed by volume re-expansion may lead to alterations in CBF and neuropathological lesions similar to GMH/IVH and IVH of choroid plexus origin.

Similarly, newborn infants experiencing perinatal asphyxia commonly suffer episodes of fetal bradycardia and acidosis; at the time of delivery they may be profoundly hypotensive and hypoxic. Although the neuroradiological and neuropathological picture of the acutely asphyxiated newborn is characteristically one of subcortical and periventricular white matter abnormalities, perinatal asphyxia causes a variety of neuropathological changes including neuronal necrosis, status marmoratus of the basal ganglia, and GMH/IVH. Studies of neonatal animal models and newborn infants with perinatal asphyxia reveal diffuse alterations in CBF and have led to the hypothesis that the neuropathological and clinical sequelae may be secondary to CBF values.

Similar to adult animal investigations, studies of fetal and newborn animals exposed to hypoxia demonstrate that this insult increases CBF. When Cavazzuti and Duffy examined the response of the newborn beagle to this insult, they found significant increases in CBF to the central and cortical gray regions but only small increases in flow to the periventricular white matter. In studies of local cerebral glucose utilization in this system, Duffy, et al., demonstrated that hypoxia produced a marked uncoupling of CBF and metabolism in the periventricular white matter with significant increases in metabolic activity and only small increases in CBF. They hypothesized that this failure of the cerebral microvasculature to provide "compensatory hyperemia" to the periventricular white matter may result in the neuropathological changes found there.

The CBF is believed to be largely independent of autonomic stimuli and controlled by local metabolic needs. Thus, hypoxia may increase blood flow to the developing brain. In the newborn beagle pup, the germinal matrix is known to be a "low-flow" zone which is neuropsychologically similar to that found in human infants of 30 to 32 weeks gestational age. Throughout 42 litters of animals, we have consistently noted the presence of "spontaneous" low-grade GMH/IVH in control pups and believe that these hemorrhages are clinically similar to those detected in newly born preterm infants at the 6th postnatal hour and may be attributed to perinatal events.

We have demonstrated significant increases in regional CBF in the brain of the newborn beagle pup exposed to normocarbic normotensive hypoxemia. The CBF was increased to cortical and central gray regions and frontal and parietal white matter but was unchanged in the parietal white matter, one of the classic sites of radiological and neuropathological injury in neonates with perinatal asphyxia. Two clinically significant and not previously reported findings were the marked increase in CBF to the periventricular germinal matrix region and the presence of GMH/IVH of choroid plexus origin in those pups exposed to hypoxemia. Unfortunately, the 14C-IAP technique does not permit determination of CBF in the choroid plexus. However, it is hypothesized that, when CBF is significantly increased, hypoxemia independent of hypertension may result in both GMH/IVH and choroid plexus hemorrhage. These data may help to explain not only hemorrhage in preterm neonates but also that

J. Neurosurg. / Volume 65 / December, 1986
found in full-term infants with hypoxemia attributable to asphyxia, congenital cyanotic heart disease, or persistent fetal circulation.

Acknowledgment

The authors gratefully acknowledge the technical assistance in this study of Ms. Dolores Montoya.

References


Manuscript received February 25, 1986.
This work was supported by Grant 11-201-856 from the American Heart Association — Connecticut Affiliate.
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