Local cerebral glucose utilization in the beagle puppy model of intraventricular hemorrhage

LAURA R. MENT, M.D., WILLIAM B. STEWART, Ph.D., AND CHARLES C. DUNCAN, M.D.
Departments of Pediatrics, Neurology, Neurosurgery, and Gross Anatomy, Yale University School of Medicine, New Haven, Connecticut

Local cerebral glucose utilization has been measured by means of carbon-14 (14C)-autoradiography with 2-deoxyglucose in the newborn beagle puppy model of intraventricular hemorrhage. Our studies demonstrate gray matter/white matter differentiation of uptake of 14C-2-deoxyglucose in the control pups, as would be expected from adult animal studies. However, there is a marked homogeneity of 14C-2-deoxyglucose uptake in all brain regions in the puppies with intraventricular hemorrhage, possibly indicating a loss of the known coupling between cerebral blood flow and metabolism in this neuropathological condition.

KEY WORDS • cerebral glucose utilization • beagle puppy • intraventricular hemorrhage

Intraventricular hemorrhage (IVH) is a major neurological problem in preterm neonates.5,24,31 Little is known of the pathophysiological mechanisms involved and the effect of IVH on the neurodevelopmental outcome of these patients. Although some information is being obtained about cerebral blood flow (CBF) in animal models involving preterm neonates with IVH and in the infants themselves, little is known about the effect on cerebral metabolism of this patient population from the profound hygrocorrhachia that occurs for many days following IVH.30,44 This study is a report of local cerebral glucose utilization (LCGU) in newborn beagle pups in which IVH has been induced by the model of hemorrhagic hypotension followed by volume reexpansion.9,12 This experimental design causes changes similar to the clinical and neuropathological findings in human neonatal IVH.

Materials and Methods

Newborn beagle pups, aged 12 to 48 hours and weighing 250 to 400 gm, were anesthetized with ketamine (0.5 ml), tracheostomized, paralyzed with 0.5 ml flaxedil, and artificially ventilated. Bilateral femoral artery and venous lines were inserted and arterial blood pressure was continuously monitored. Arterial blood gases were frequently checked, and ventilatory volume or rate was adjusted to maintain PO₂ at 40 to 60 torr and PCO₂ at 31 to 40 torr prior to the initiation of IVH. Temperature was monitored via a rectal probe, and animals were kept under warming lamps to maintain body temperature.

In the experimental animal group, 20% to 25% of the estimated blood volume was withdrawn over 1 to 2 minutes into a heparinized syringe. After 5 minutes of hemorrhagic hypotension, the blood was rapidly reintroduced via the femoral venous catheter. The animals were then observed for 45 minutes, following which time they received 200 μCi/kg of carbon-14 (14C)-2-deoxyglucose (2DG) via the femoral venous catheter. They were observed for an additional 45 minutes, then were rapidly sacrificed with an overdose of sodium pentobarbital. The brains were quickly removed and frozen in isopentane at -60°C. The brains were then prepared for 14C-autoradiography. Sections were cut at 32 μ in a cryostat. Every 25th section was saved and placed on a glass slide; these were rapidly dried at 70°C and then placed sequentially in a cassette loaded with Kodak SB-5 film and Amersham 14C standards for 5 days.

Control animals underwent the same medication regimen, tracheostomy and ventilation routine, and line placement as the experimental group but did not undergo the hemorrhagic hypotension or reinfusion manipulations. Like the experimental group, they re-
Cerebral glucose utilization in intraventricular hemorrhage

TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Dogs</th>
<th>MABP (mm Hg) Before Trough Peak</th>
<th>Pathology</th>
<th>14C-2DG Concentrations (μCi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cortex</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unanesthetized</td>
<td>3†</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(27-36)</td>
<td>18</td>
</tr>
<tr>
<td>anesthetized</td>
<td>6‡</td>
<td>80 throughout (65-95)</td>
<td>no hemorrhage</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(15-32)</td>
<td>22</td>
</tr>
<tr>
<td>experimental</td>
<td>6‡</td>
<td>85 (60-97)</td>
<td>GMH or IVH in all</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>71 (50-85)</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96 (80-105)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* GM = germinal matrix; GMH = GM hemorrhage; IVH = intraventricular hemorrhage; MABP = mean arterial blood pressure. Numbers in parentheses indicate range.
† Six hemispheres were analyzed.
‡ Ten hemispheres were analyzed for 14C-autoradiography.

Received 14C-2DG and were observed for 45 minutes. They were rapidly sacrificed and their brains were prepared for autoradiography as in the experimental group. An additional group of three animals received no anesthesia, were not tracheostomied, and did not have line placement. These animals received 200 μCi 2DG/kg intraperitoneally and were allowed to remain with the dam for 60 minutes. They were then rapidly sacrificed and their brains were prepared for 14C-autoradiography as described above.

The 14C-2DG concentrations were determined densitometrically from calibrated plastic standards. Values were obtained for six regions of the cortex, six corresponding regions of hemispheric white matter, three regions of caudate nucleus, and three regions of germinal matrix in autoradiographs corresponding to the section at the head of the caudate nucleus. Since in this preliminary study blood glucose concentrations were not assayed, it is not possible to provide absolute values for LCGU. However, if one assumes nearly identical arterial blood-time curves and similar lumped constants among the animals in all of the groups and between groups, then the concentrations of 14C-2DG should reflect the relative LCGU for the regions.

Results

Gross pathological and microscopic evidence was found for either germinal matrix hemorrhages or IVH in all of the experimental animals but in none of the control pups.

Densitometric evaluation of the 14C-autoradiographs of the unanesthetized control group yielded mean concentrations of 32 μCi (range 27 to 36 μCi) for the cortex, 18 μCi (range 16 to 19 μCi) for the hemispheric white matter, 24 μCi (range 20 to 27 μCi) for the caudate nucleus, and 19 μCi (range 16 to 21 μCi) for the germinal matrix. As might be expected from studies with adult animals, the concentrations of 14C in the cortex were higher in the unanesthetized control group than in the anesthetized control ani-
ing gestational age appears to predispose to IVH, and, in our Newborn Special Care Unit, approximately 50% of all infants of less than 1250 gm birth weight who survive longer than 36 postnatal hours experience IVH. Numerous investigators have noted the clinical association of IVH and hypercarbia, hypoxia, large MABP shifts, and volume changes in small preterm infants. Wigglesworth and Pape have advanced the hypothesis that IVH may be a manifestation of alterations on CBF for the developing brain. Indeed, it is the germinal matrix region, with its numerous capillaries but little glia, myelin, or supporting structures, which has been described as the watershed zone of the preterm cerebrum which may be most susceptible to changes in CBF.

Although IVH may now be readily detected by computerized tomography (CT) or cranial echoencephalography, and the course of disappearance of the hemorrhage and frequent dilatation of the ventricular system which may follow this insult may also be observed, physicians caring for these infants must also worry about the long-term neurodevelopmental outcome of these patients. Intraventricular hemorrhage, bloody cerebrospinal fluid (CSF), and CT or echoencephalographic findings are simply a result of changes in blood flow to both the germinal matrix

---

**Fig. 1.** Coronal section of a control brain and its autoradiograph are shown in A and B, respectively. Coronal section of a brain with intraventricular hemorrhage is shown in C, and the $^{14}$C-2-deoxyglucose (2DG) autoradiograph demonstrating relative homogeneity of 2DG utilization is depicted in D.
Cerebral glucose utilization in intraventricular hemorrhage

and cortical structures, and patients with the more severe grades of IVH are known to experience significant neurodevelopmental handicaps, including mental retardation, hemiparesis, and seizures. Others, with more minor hemorrhages, have been found to have learning disorders, behavior changes, and CT evidence of cerebral atrophy. As in adults with subarachnoid hemorrhage (SAH), hypoglycemia (low CSF sugar levels) is found in most neonates with IVH, and both the etiology of this problem and its implication for the developing brain are unknown. Indeed, little is known about the metabolism of the immature cerebrum. Vannucci, et al., studied six preterm neonates with obstructive posthemorrhagic hydrocephalus, and reported significant decreases in ventricular CSF glucose values in association with elevations in lactate and lactate/pyruvate ratios, despite normal peripheral values, compared to five infants with congenital hydrocephalus secondary to other causes. These authors therefore dismissed the possibility that these metabolic changes were the result either of the presence of residual red or white blood corpuscles or of changes in the blood-brain barrier secondary to the hemorrhage or to the cellular breakdown products. They hypothesized that these metabolic findings were due to altered oxidative metabolism of the immature brain.

Kennedy, et al., have described the CBF to the developing brain. Recently Goddard, et al., discovered that the newborn beagle pup is a model for IVH, with hemorrhage originating in the germinal matrix tissue in response to hypercarbia, relative hypotension followed by reperfusion, or acute hypertension similar to the preterm neonatal brain. This model has provided an excellent resource for the study of cerebral metabolism in IVH. The investigations of Goddard, et al., and Pasternak, et al., and our own studies have noted that the germinal matrix is a relatively low-flow region and that CBF selectively increases to this region in the presence of IVH, thus indicating focal changes in CBF with this model.

In the normal brain, CBF and cerebral metabolic rate (CMR) are believed to be tightly coupled. However, in the pathological condition, this relationship may be lost. In the presence of chemically induced seizures in both adult dogs and monkeys, Plum, et al., described an increase in CBF of 260%, while CMR increased by only 60%. In addition, several investigators have reported increased oxygen metabolism in experimental animals with generalized ischemia or SAH, Fein reported a primary depression of cerebral glycolysis with normal stores of energy-rich phosphates and normal CBF in adult monkeys with controlled SAH.

Like CBF, CMR appears to be lower in newborn animals than their adult counterparts, and although Gardiner could detect no overall alterations in the CMR of glucose in both fetal lambs and calves exposed to brief periods of hypoxia and prolonged asphyxial episodes, Kjellmer, et al., reported decreased CMRO₂ in fetal lambs with hypoxia, and Vannucci and Duffy, studying high-energy phosphate compounds in the whole brain of neonatal beagle puppies, detected significant decreases in cerebral glucose, G6P and F6P, with corresponding marked increases in pyruvate and lactate indicative of accelerated glycolytic flux into anaerobic pathways.

The recent development of the ¹³C-2DG technique by Sokoloff and coworkers for studying local cerebral glucose utilization (LCGU) has permitted the investigation of flow and metabolism in very discrete regions of brain. As in whole brain, Jones, et al., demonstrated the linear relationship between CBF and CMR in paired brain regions. However, because of the marked heterogeneity of LCGU in the normal brain, one might expect heterogeneity of response of LCGU to pathophysiological insult. When Miyaoka, et al., studied the effects of graded hypoxemia on LCGU in the awake rat, no overall change of the CMR of glucose was found, but marked and widespread focal changes of LCGU were demonstrated.

Hemorrhagic hypotension, the first stage in the pathophysiological insult that we have used to produce IVH in the newborn pup, has been studied in the adult rat. Widespread alterations in LCGU were noted, and while Greenberg, et al., reported decreased LCGU in cortical regions, Savaki, et al., noted an increase in LCGU in 10 other brain regions including diencephalic, midbrain, and brain-stem structures. In addition, when Pulsinelli and Duffy determined LCGU in adult rats exposed to graded hypoxemia, they reported marked increases in LCGU in the periventricular white matter.

In summary, we have found ¹⁴C concentrations of 2-deoxyglucose (2DG) to be representative of LCGU in newborn beagle pups exposed to the relative hypotension/reperfusion model of IVH. Although changes in CBF are known to be focal to the germinal matrix, we noted changes in the ¹⁴C-2DG concentrations throughout the cerebral hemispheres with uncoupling of metabolism and flow and the attainment of relative homogeneity of LCGU throughout.

References

Cerebral glucose utilization in intraventricular hemorrhage


Manuscript received January 27, 1982.
This work was sponsored in part by the Charles H. Hood Foundation, Boston, Massachusetts and the Ohse Fund of the Department of Surgery, Yale University School of Medicine, New Haven, Connecticut.
Address reprint requests to: Laura R. Ment, M.D., Department of Pediatrics, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.