Differences in critical cerebral blood flow with age in swine

JUN HARADA, M.D., AKIRA TAKAKU, M.D., SHUNRO ENDO, M.D., NAOYA KUWAYAMA, M.D., AND OSAMU FUKUDA, M.D.

Department of Neurosurgery, Toyama Medical and Pharmaceutical University, Toyama, Japan

- Normal cerebral blood flow (CBF), critical CBF at a flat reading of the electroencephalogram (EEG), and reversibility of the flat EEG after reperfusion were investigated in a total of 59 pigs, including 7 newborns (1 to 3 days of age), 38 juveniles (1 month old), and 14 adults (7 months old). The CBF was determined by the hydrogen clearance method; the EEG was recorded continuously and a power spectrum analysis was performed. Cerebral ischemia was produced by occlusion of both common carotid arteries and induction of hypotension (approximately 50 mm Hg). The flat EEG reversibility was investigated for 3 hours after reperfusion. As parameters of brain development, the neuronal density and the time at which the S-100 protein appeared in the brain were examined.

- Normal CBF was highest in neonatal pigs and decreased with age. The critical CBF at a flat EEG was lowest in newborn pigs and was elevated with development of the brain. Tolerance against cerebral ischemia was greatest in newborn pigs.

Key Words • cerebral blood flow • ischemia • S-100 protein • brain growth • swine

Children sometimes exhibit a surprising ability to recover from neurological impairments caused by cerebrovascular events. On the other hand, once irreversible damage has occurred, they suffer severe sequelae such as delays in mental and motor development. Clarification of the critical levels for ischemia in the developing brain is indispensable in an effort to better understand cerebrovascular disorders in children.

Many reports, both clinical and experimental, have been published on the critical level of cerebral blood flow (CBF) during cerebral ischemia. Electrical failure of the brain is generally considered to occur at a CBF of 15 to 18 ml/100 gm/min and energy failure to occur at 10 ml/100 gm/min. However, these values have been estimated mainly from mature brain and depend on the anesthetic agents used. Several authors have evaluated the critical level of CBF in the developing brain, whereas few have made comparisons with mature brain using the same method and a similar specimen.

The duration of ischemia is also an important factor in determining the reversibility of brain dysfunction. The critical CBF level causing electrical failure is considered to be approximately 10 ml/100 gm/min in the adult brain; however, the critical level in the developing brain remains unknown. In the present study two factors, the threshold of CBF and the duration of ischemia, were evaluated by means of the hydrogen clearance method and electroencephalography (EEG) in the immature and mature brain of pigs.

Materials and Methods

A total of 59 pigs were studied, including seven neonates aged 1 to 3 days, 38 juveniles aged 1 month (weaning age), and 14 adults aged 7 months (sexual maturation completed). These groups ranged in body weight from 1 to 2 kg, 4 to 7 kg, and 100 to 120 kg, respectively. The mean brain weight for each group was 30, 60, and 120 gm, respectively.

Preparation of Ischemia Model

After the induction of anesthesia with intramuscular ketamine hydrochloride (50 mg/kg) and intravenous phenobarbital (10 mg/kg), a tracheotomy was performed. The animals were immobilized with pancuronium bromide, and the PaCO₂ was adjusted to between 30 and 40 mm Hg under controlled ventilation. The femoral artery was catheterized for measurement of blood pressure and blood gas analysis. Bilateral common caro-
tid arteries were isolated. Cerebral ischemia was produced by a combination of bilateral common carotid artery occlusion using silk thread and the induction of hypotension to about 50 mm Hg with trimethaphan camphosulfonate. The brain was reperfused after random intervals. Carbon was perfused through the common carotid arteries 3 hours after reperfusion to confirm the absence of thrombosis.

**Measurement of CBF and EEG Recording**

A small craniotomy, 1 cm square, was made in the left parietal region. A platinum needle electrode was inserted to a depth of about 2 mm into the left parietal cerebral cortex, and the cortical blood flow was determined by the inhalation hydrogen clearance method before and during ischemia, and after reperfusion. The EEG recording was made continuously with bipolar leads in the bilateral parietal regions until 3 hours after reperfusion; an analysis of the power spectrum was then performed.

The CBF was determined to be critical when the animals showed a flat EEG recording during ischemia. The animals were divided into three groups according to EEG recovery: complete recovery, incomplete recovery, or no recovery.

**Evaluation of EEG Reversibility after Reperfusion**

The relationship of CBF during ischemia and the duration of ischemia to EEG reversibility were analyzed statistically. The CBF levels during ischemia and the duration of ischemia were plotted separately for complete recovery versus incomplete or no recovery in the newborn, juvenile, and adult animal groups. In the juvenile group, the cut-off line between the range of values for the two recovery groups was determined using a discriminant function method. A quadratic equation was used for this determination: \( F = a \times T^2 + b \times T + c \), where \( F \) is the CBF during ischemia and \( T \) the duration of ischemia. To minimize errors, this statistical procedure was applied only to the juvenile group, which comprised the largest number of animals. The EEG reversibility among newborn, juvenile, and adult pigs was compared by the logistic regression method.\(^9\)

**Histological Evaluation**

Neuronal density and the appearance of S-100 protein were examined as parameters of brain development in each age group. Neuronal density and the presence of S-100 protein in the parietal cerebral cortex were determined by immunohistochemical staining of neuron-specific enolase and alpha-subunit of S-100 protein.

**Results**

**Normal CBF Measurements**

The normal CBF was 47.7 ± 12.8, 43.6 ± 8.3, and 27.0 ± 4.7 ml/100 gm/min (mean ± standard deviation) in newborn, juvenile, and adult pigs, respectively (Fig. 1). Significant differences (p < 0.01) were observed between the newborn and adult groups and between the juvenile and adult groups. No significant differences were seen between the newborn and juvenile pigs based on one-way analysis of variance.

**Threshold of CBF for EEG Flattening**

The critical CBF that caused EEG flattening was 7.2 ± 0.9, 8.8 ± 3.4, and 12.7 ± 1.8 ml/100 gm/min in newborn, juvenile, and adult pigs, respectively (Fig. 2). Significant differences (p < 0.05) were observed between the newborn and adult groups and between the juvenile and adult pigs, while no significant differences were found between the newborn and juvenile pigs using one-way analysis of variance.

**Statistical Analysis for Critical CBF**

**Discriminant Function Method (Juvenile Group Only).** The cut-off line for EEG reversibility following reperfusion was determined by the discriminant function method in juvenile pigs (Fig. 3 and Table 1). According to the equation described above, CBF during ischemia (\( F = -5.3 \times 10^{-4} \times T + 0.21 \times T - 3.8 \)). Thus, EEG flattening was always reversible when the reduced CBF was 17 ml/100 gm/min or above, regardless of the duration of ischemia. The discriminant function method demonstrated that when CBF was reduced to approximately 10 ml/100 gm/min for 80 minutes or longer, EEG recovery was probable for 77% of the juvenile group.
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**Fig. 2.** Cerebral blood flow (CBF) threshold at which the electroencephalogram became flat in newborn, juvenile, and adult pigs. The CBF threshold was elevated with development of the brain. Significant differences were observed between the newborn and adult groups and the juvenile and adult groups, but not between the newborn and juvenile groups. *Asterisks = p < 0.05. n = number of animals in each group.*

**Logistical Regression Method.** As the discriminant function method has an increasing possibility of errors in comparing groups with small populations (newborn and adult; Figs. 4 and 5), the logistical regression method was used for comparisons among all three groups (Table 2). The analysis revealed EEG reversibility to be significantly higher in newborn pigs than in juvenile or adult pigs (odds ratio = 7.90, p = 0.09). In other words, the newborn group appeared to survive a longer duration of ischemia and a lower CBF level than the other two groups.

**Neuronal Density and Brain Development**

The neuronal density decreased with further development of the brain by a ratio of 2.5:2:1 for newborn, juvenile, and adult pigs, respectively. The S-100 protein was observed in the newborn pigs and showed no qualitative changes with the development of the brain.

**Fig. 3.** The relationship of cerebral blood flow (CBF) during ischemia and the duration of ischemia to reversibility of a flat electroencephalogram (EEG) at 3 hours after reperfusion in juvenile pigs. *Open circles represent complete recovery of EEG and closed circles depict incomplete or no recovery of EEG at 3 hours after reperfusion. The solid line was obtained by a discriminant function calculation, see text. The broken line indicates data obtained from earlier reports. When the CBF during ischemia was 17 ml/100 gm/min or greater, the EEG recovered after reperfusion, regardless of the duration of ischemia. When CBF fell to about 10 ml/100 gm/min for more than 80 minutes, certain reversibility of EEG was lost.*

**Fig. 4.** The relationship of cerebral blood flow (CBF) during ischemia and the duration of ischemia to reversibility of a flat electroencephalogram at 3 hours after reperfusion in newborn pigs. *Open and closed circles are as defined in Fig. 3.*

**Fig. 5.** The relationship of cerebral blood flow (CBF) during ischemia and the duration of ischemia to reversibility of a flat electroencephalogram at 3 hours after reperfusion in adult pigs. *Open and closed circles are as defined in Fig. 3.*

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TABLE 1  
Classification of EEG recovery by discriminant function method

<table>
<thead>
<tr>
<th>EEG Outcome</th>
<th>Estimated Complete Recoveries</th>
<th>Estimated Incomplete &amp; No Recoveries</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>true complete recovery</td>
<td>11</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>true incomplete + no recovery</td>
<td>3</td>
<td>17</td>
<td>20</td>
</tr>
</tbody>
</table>

* Equation obtained by discriminant function method was as follows: F = -5.3 × 10^7 × X^2 + 0.21 × T - 3.8, where F = CBF during ischemia and T = occlusion time. Probability = (11 + 17)/(16 + 20) = 0.7778. EEG = electroencephalographic flattening.

Discussion

Clinical and experimental studies concerning the critical level of CBF and the resistance of developing brain to ischemia have been few in comparison to studies of the mature brain. Among the reasons for the lack of studies are: clinically, the difficulty in obtaining cooperation for CBF measurements in children and, experimentally, the small size and vulnerability of the brain to surgical insult in immature animals.

Animal Model

We chose pigs as the experimental model in this study because their brains are of sufficient size in the neonatal period, their intracranial vascular architecture is relatively similar to that of humans, and their rate of increase in brain weight with age is nearly identical to that of humans. In addition, occlusion of bilateral carotid arteries and intentional hypotension produce various levels of ischemia, which was considered to be ideal for the purpose of this study. The drawbacks include heavy body weight (> 100 kg) and a very thick, hard cranium.

Normal Cerebral Blood Flow and Age

In general, the normal CBF in humans is said to be low at birth but increases rapidly to reach a peak between 1 and 5 years of age; thereafter, CBF decreases with age. Kety stated that the decrease in CBF was nearly parallel to the decrease in neuronal density observed with aging. However, the rapid increase in CBF from the neonatal period to infancy and early childhood cannot be explained solely by the increasing metabolic demand of neurons. Myelination of the brain or proliferation and maturation of glial cells must be also considered as factors in the increasing demand of CBF.

Several studies in which the CBF of newborn infants was measured by venous occlusion plethysmography have shown CBF to be lower in infancy than in early childhood. In our study, the CBF in pigs was highest in the newborn group (1 to 3 days of age), perhaps because the brain of a newborn pig has developed enough to be capable of ambulation immediately after birth. The high neuronal density in the newborn pig may also be one of the factors explaining the highest demand of CBF at that age.

Critical Cerebral Blood Flow and Age

Although there have been a number of reports about the critical levels of CBF in adult brains, few reports compare the two age groups using the same method and a similar specimen. In the present study, the mean critical CBF levels in newborn and juvenile pigs (7.2 ± 0.9 and 8.8 ± 3.4 ml/100 gm/min, respectively) were significantly lower than that found in the adult group. These results indicate that the critical CBF level at the time of a flat EEG tracing is elevated with age. Since the hydrogen clearance method is not completely reproducible when CBF falls below 5 ml/100 gm/min, the critical values in this study may not be sufficiently reliable and another method may be needed to evaluate the critical level of CBF.

There have been detailed reports on cerebral protection by anesthetics. In the present study, pentobarbital was used as the anesthetic agent; therefore, the critical CBF determined here might be low because of the use of a barbiturate.

Reperfusion Recovery

Whether the impaired functions of the brain will recover after reperfusion is one of the most important problems in clinical and experimental studies of brain ischemia. The reversibility of brain dysfunction is affected by factors such as the CBF during ischemia, duration of ischemia, blood pressure after reperfusion, and the area of ischemia. Among these, the CBF during ischemia and duration of ischemia are considered to be the most important factors. There is a difference between critical CBF and neuronal death; the greater the reduction of CBF during ischemia below critical CBF levels, the shorter the period in which neural tissue will tolerate ischemia.

There have been detailed reports concerning CBF during ischemia and duration of ischemia in various models of experimental ischemia. According to Jones, et al., adult monkeys exhibited reversible motor paresis when CBF was reduced below 23 ml/100 gm/min by occlusion of the middle cerebral artery; irreversible cerebral infarction occurred when CBF was reduced to 17 or 18 ml/100 gm/min permanently or 10
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to 12 ml/100 gm/min for 2 to 3 hours. In an adult cat model of whole-brain ischemia, Carter et al. reported that the CBF threshold for a flat direct cortical response was 8.7 ± 3.4 ml/100 gm/min. If that rate was maintained for 1 hour, the direct cortical response became irreversible.

A comparison between reversibility in mature and developing brains in the same ischemia model has not been previously reported. In this study, variations in the reversibility of brain failure were examined in pigs ranging in age from 1 day to 7 months. In juvenile pigs, the reversibility of flat EEG tracings after reperfusion was lost when the CBF was reduced to about 10 ml/100 gm/min for 80 minutes. A comparison between the newborn and juvenile groups by a logistical regression model indicated that the EEG flattening in newborn pigs tended to be more readily reversed at a lower CBF than after a longer duration of ischemia. However, the reversibility of the EEG abnormality was not significantly different between the juvenile and adult groups. The newborn pig was the most resistant to cerebral ischemia.

Significance of S-100 Protein

In the human, S-100 protein is said to appear in the frontal lobe relatively late (at about 40 days after birth). Assuming the S-100 protein to be a parameter of brain development, we investigated the time of its appearance. It was already present in the brain of the newborn pig as well as in the other groups in our qualitative assay.

References


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Address reprint requests to: Jun Harada, M.D., Department of Neurosurgery, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama, Japan.