Effect of ischemia on metabolism of the brain of the newborn mouse

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THURSTON, JEAN HOLOWACH, and DAVID B. McDOUGAL, JR. Effect of ischemia on metabolism of the brain of the newborn mouse. Am. J. Physiol. 216(2):348-352. 1969.—Changes in six metabolites in the brains of newborn mice were followed during the ischemia induced by decapitation. Phosphocreatine fell rapidly, glucose and glucose 6-phosphate more slowly, and ATP and glycogen were the most stable of the substances measured. From the changes, the initial rate of use of high-energy phosphate (cerebral metabolic rate) was calculated to be 2.3 mmoles/kg per min, which is .09 of the adult rate following decapitation. Lactate accumulated much more slowly than in the adult. In the early stages (first 4 min) of ischemia the rate of disappearance of energy reserves was nearly linear, and much faster than the rate in the later stages of the experiment. The energy reserves of the brain are about the same in young as in adult animals. In view of this, and the low rate of glycolysis, it is concluded that the chief factor in the resistance of very young animals to ischemia is the low cerebral metabolic rate, not high glycolytic capacity.

Changes in cerebral ATP, P-creatine, glucose, glucose-6-P, glycogen and lactate during anoxia

Compared to older animals, the newborn of all mammalian species show greater resistance to oxygen lack (6, 7, 9, 12). The physiologic basis of this resistance is still not completely understood, but has often been ascribed to a greater dependence on glycolytic metabolism. Today microanalytical methods of great sensitivity and specificity permit a new biochemical approach to this problem. Using such techniques, Lowry and co-workers (13) studied the effects of ischemic anoxia on the brain in adult and 10-day-old mice. By decapitation they converted the brain to a closed, anoxic system in which the rates of change of all major sources of energy could be followed. They found a lower metabolic rate in the brains of the 10-day-old animals. Glycolysis was also slower. The present study was undertaken to make a similar appraisal of cerebral energy use rate and glycolytic rate in the newborn. Since the experimental methods used are similar, the results can be compared directly with those for older animals (13). This comparison has led to clarification of the role of glycolysis during early development.

METHODS

Preparation of animals. The study comprised six litters with a total of 47 newborn Swiss-Webster albino mice. At birth some mice exhibit apnea and cyanosis. Experiments were not begun till regular respirations were established and the color had returned to normal, either spontaneously or as a result of maternal stimulation. For each of the animals in five of the six litters, experimental procedures were completed within 4 hr after birth. The age of the sixth litter is not known so precisely, but was not more than 12 hr. The biochemical findings in this litter were indistinguishable from the others and are included in the experimental results.

After decapitation the heads were kept at 37 C for measured intervals and then frozen in Freon 12 (CCl,F) at its melting point (−150 C) with rapid stirring. Zero-time animals were frozen whole. Prior to preparation of tissue extracts, the specimens were kept at −70 C.

Preparation of tissue extracts. The method of brain dissection and tissue extraction was as described by Lowry and associates (13). Briefly the procedure is as follows. The brain was dissected from the animal at −20 C and ground to a powder with a mortar and pestle chilled with liquid nitrogen. The brain powder was weighed at −20 C. The brain samples (25 60 mg) were placed over 0.163 ml of frozen 3 M HClO4 in plastic tubes surrounded by Dry Ice. Brain powder and perchloric acid were then homogenized for 10 min with a glass stirring rod in an alcohol bath at −10 C (at this temperature 3 M HClO4 is a liquid). To each tube 0.666 ml of 1 mM ethylenediaminetetraacetic acid (EDTA) was added at 4 C. The supernate (0.75 ml) was neutralized...
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with 0.163 ml of 2 M KHCO₃. Extracts were stored at -70 C until the time of assay.

Assay methods Enzymatic methods were used to measure ATP, phosphocreatine, lactate, glucose, and glucose 6-phosphate (13) and glycogen (16).

Calculations. Following the example of Lowry et al. (13), actual and potential molar equivalents of high-energy phosphate were calculated as 1 equivalent of high-energy phosphate for each mole of P-creatine, 2 for each mole of ATP and glucose, 2.9 equivalents for each glucose equivalent of glycogen, and 3 for each mole of glucose-6-P.

RESULTS

Initial metabolite levels. The initial levels of the substances measured in the present study are shown in Table 1 together with data for 10-day-old and adult animals obtained by Lowry et al. (13). In the newborn mice, initial brain glucose was quite variable, ranging from nearly 0 to a high of 1.67 mmoles/kg. Glucose-6-P was relatively high in the newborn, twice that found in older animals. Initial lactate values were higher in newborn mice than in 10-day-old animals. Variations in initial brain lactate may be a reflection of the fact that at birth the degree and duration of cyanosis differ in each mouse. Total cerebral reserves of ATP, P-creatine, glucose, glucose-6-P, and glycogen, calculated in terms of molar equivalents of high-energy phosphate, were similar in all three age groups.

Changes in metabolite levels during ischemia. (In the material which follows comparisons are made with data for 10-day-old and adult animals obtained by Lowry et al. (13).) In the newborn the changes in metabolites following decapitation were much slower than in the adult or 10-day-old mice. The changes in ATP are a dramatic example of the age-dependent differences (Fig. 1). After 2 min, the ATP level in the newborn was unchanged, while the level had fallen to 20 % in 10 day old animals and 10 % in adults. Even after 4 min, ATP in the newborn was still 70 % of the initial value.

Of the substances measured P-creatine fell most rapidly (Fig. 2), as it does in older animals, but the drop in the first 30 sec was only one-half of that in older animals.

By 1 min, glucose had dropped 30 % in the newborn (Fig. 2), while in half this time glucose had fallen 60 % in 10-day-old mice and 80 % in adults. Glucose-6-P fell to one-half in 30 sec and to one-third at 1 min. With continued anoxia no further significant changes were seen.

Since in the adult mouse brain glycogen falls 90 % in the 1st min after decapitation, the stability of brain glycogen in the young animals is truly remarkable. In

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Newborn (11 mice)</th>
<th>10-day-old mice*</th>
<th>Adult*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-creatine</td>
<td>3.4±0.5</td>
<td>3.2</td>
<td>2.4</td>
</tr>
<tr>
<td>ATP</td>
<td>2.25±0.16</td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>Glycogen</td>
<td>2.35±0.26†</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.88±0.17</td>
<td>0.75</td>
<td>1.54</td>
</tr>
<tr>
<td>Glucose-6-P</td>
<td>0.70±0.01</td>
<td>0.09</td>
<td>0.08</td>
</tr>
<tr>
<td>Lactate</td>
<td>2.7±0.5</td>
<td>1.6</td>
<td>2.3</td>
</tr>
<tr>
<td>~P₂</td>
<td>17.0</td>
<td>17.7</td>
<td>17.0</td>
</tr>
</tbody>
</table>

Values for newborn are means ± SE. * Taken from Lowry et al. (13). † This value is the mean for 19 brains. ‡ ~P₂ represents total high-energy phosphate reserves, calculated as explained in METHODS.

FIG. 1. Effect of ischemia on ATP levels in the brain of newborn, 10-day-old, and adult mice. In the newborn each value represents the mean of 3-12 animals (total 46 mice). In this and in Figs. 2 and 3, vertical lines represent ±1 standard error. Data for the older animals are taken from Lowry et al. (13).

FIG. 2. Concentrations of P-creatine, glucose, glucose 6 P, glycogen, and lactate in the brain of newborn mice frozen at different intervals after decapitation. Vertical lines are as in Fig. 1. Except for glucose-6-P, each point represents the mean value for 4-12 animals (total 47 mice). Glucose-6-P value in zero-time animals is the average for 7 mice. At later times 2 or 3 mice are represented. At the scale of the figure glucose-6-P values are difficult to represent; they were, respectively, 0.200, 0.097, 0.070, 0.050, 0.047, 0.068, and 0.050 mmoles/kg.
newborn and 10-day-old animals, the rate of fall was about equal and very slow for the first 4 min. However, at later times, the rates of use of glycogen became markedly different. After 10 min, when glycogen in 10-day-old animals had virtually disappeared, one-half was still present in the newborn (Fig. 2). Even after 20 min one-third remained.

In the newborn, brain lactate rose slowly after an initial short lag (Fig. 2). The balance between glucose and glycogen loss and lactate gain was quite close throughout the experiment.

The disappearance of energy reserves was nearly linear for the first 4 min of ischemia, and much faster than the rate at later times (Fig. 3). The initial rate of energy use (the cerebral metabolic rate) in the newborn was calculated to be 2.3 mmoles high-energy phosphate equivalents/kg per min. Corresponding rates for the 10-day-old and adult mice are 13 and 25 mmoles/kg per min, respectively. Assuming that the rate of use was unchanged immediately after decapitation and making the other assumptions made by Lowry et al. (13), the rate of glucose use in the newborn before decapitation was only 0.07 mmoles/kg per min. After the onset of anoxia, the maximal rate at which glycolysis proceeds is, as expected, much faster (Table 2). To maintain anaerobically the same rate of high-energy P generation, the glycolytic rate would need to increase about 17-fold. In fact, however, the rate in newborn and adult increased only 8–10 times and in 10-day-old mice only 4 times (Table 2).

**DISCUSSION**

The sequence of changes in the major energy reserves of the newborn mouse brain is much the same as it is in the adult (13). Thus, P-creatine falls most rapidly, ATP and glycogen least. However, the rate at which the changes occur is much slower, and this is, of course, a reflection of the slow-energy use rate. The conclusion is that in newborn mouse, the cerebral metabolic rate is very slow, 0.09 of that found in the adult (13). After the first 4 min the metabolic rate falls even lower so that between 10 and 20 min the rate is .07 of the initial rate. There appears to be little reason to suppose that the need for energy has fallen to this extent, but it may be that energy supplies have become less accessible, perhaps because of a drop in intracellular pH. Such an explanation has been proposed, in more detail, for the decreasing metabolic rate in the ischemic brains of poikilotherms (14). From the changes in cerebral ATP levels during anoxia in rats pretreated with iodoacetate, Samson, Balfour, and Dahl (17) also deduced that energy use was much slower (.08) in 1-day-old rats than in 21-day-old rats. And Greengard and McIlwain (8) showed that O2 consumption by surviving stimulated rat cerebral slices was three times greater in adults than in newborn. The metabolic rate for stimulated slices of newborn brain was about the same as that found in the present experiments. However, the rate for adult brains was only one-half of that found in adult mice (13) or in 21-day rats (17). It appears, therefore, that even with electrical stimulation it may not be possible to raise the activity of isolated slices to the level maintained by the intact adult brain.

It has long been known that infant animals survive without oxygen much longer than do adults (see Dawes (4) for a comprehensive review). Himwich et al. (10) showed that the survival of newborn rats in an atmosphere of nitrogen, and the duration of gasping in the isolated head, were much shortened by pretreatment of the animals with iodoacetate. This was confirmed by

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**TABLE 2. A comparison of pre- and postdecapitation hexose flux rates, energy use, and energy production in mice at three different ages**

<table>
<thead>
<tr>
<th></th>
<th>~P Use</th>
<th>Calculated</th>
<th>Maximal Glucose</th>
<th>~P Production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Predecapitation</td>
<td>or Glucose Plus</td>
<td>From Maximal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glucose-Use Rate</td>
<td>Glycogen Use</td>
<td>Glycolysis</td>
</tr>
<tr>
<td>Newborn</td>
<td>2.3</td>
<td>0.07†</td>
<td>0.7</td>
<td>1.7</td>
</tr>
<tr>
<td>10-day-old</td>
<td>13</td>
<td>0.4</td>
<td>1.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Adult</td>
<td>25</td>
<td>0.76</td>
<td>6.5</td>
<td>13</td>
</tr>
</tbody>
</table>

*Glucose disappearance only is used for adult; average initial glucose flux plus average initial glycogen flux are used for 10-day-old (1st 0.5 min (13), and for newborn mice (1st 2 min, Fig. 2). † These figures are obtained by multiplying the maximal glucose use (13) by 2 for the adult and by adding 2 times the average initial glucose flux and 2.9 times the average initial glycogen flux for 10-day-old and newborn mice, calculated from data for the 1st 0.5 min for 10 day (13) and for the 1st 2 min for newborn mice (see Fig. 2). It is assumed that a) the rates of ~P use before decapitation were unchanged in the first few minutes after decapitation, b) glucose was the only source of energy before decapitation, c) 15% of the glucose used was converted to lactate, as in the adult (15, chapt. 2), and d) the remaining glucose was oxidized to CO2 with a yield of 30 equivalents ~P/mole.

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**Fig. 3. Total cerebral energy reserves (see text) at different intervals after decapitation in newborn mouse. Values are based on the data of Figs. 1 and 2.**
Samson and Dahl (9) and by Jilek and Trojan who also showed that the treatment with iodoacetate had much less effect on the persistence of gasping in the isolated heads of adult animals (cited in 11). Results of this sort have been interpreted as indicating that glycolysis is more important in immature brains than in adults. In a recent text (13, p. 289), for example, the following statement is found: "Glycolysis appears however to playa more critical role as energy yielding reaction in the early stages of mammalian development. After birth, respiration increases until its contribution greatly preponderates."  

Such interpretations persist despite the fact that the explanations given by the experimenters themselves were quite different. In their study of survival in anoxia and ischemia with and without iodoacetate, Himwich et al. (10) also showed that lactate production was much slower in excised cerebral tissues of the newborn than in those of the adult. Similarly, Chesler and Himwich (3) found that glycolysis was 0.25-0.2 as fast in the newborn with interference with the supply of oxygen glycolysis as in adult rat brain, and later Grcengard and McIlwain (11) showed that lactate production was much lower in excised cerebral tissues of the newborn than in adults (unpublished experiments). Therefore the metabolic rate may be a major determinant of survival time, but other factors may also contribute.

One such factor seems to be the circulation. Survival (time to last gasp) is longer in intact anoxic animals than in decapitated heads (for review, see Dawes (4)). This is reasonable, and probably means that glucose supplies brought to brain by the circulating blood are sufficient to prolong significantly maintenance of cerebral energy levels. This would be of greater relative benefit when the metabolic rate is low, as in the newborn. A consideration of this and other parameters will be the objective of future investigations.

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REFERENCES


12. KARAT, H. The greater resistance of very young animals to anaerobic conditions glycolysis can greatly prolong survival in the newborn, is that the energy demand is so low. The difference in energy use rates between newborn and adult is presumably due to differences in structure and function. In the newborn the neurons are smaller and less highly branched, thus presenting less functional surface than in the adult. The number of synapses is known to increase with age (1), and in newborn cerebrum many neurons are not yet functional. Increase in the number of functional neurons and in the size and degree of branching of each neuron with maturation are reflected in electrolyte changes (19), and presumably these contribute to the increased energy demand found in adult brain. The question remains whether the 11-fold difference in the cerebral metabolic rates between newborn and adult mice is quantitatively great enough to account for the difference in survival times in anoxia. The evidence suggests that it is not. Newborn mice survive in an anoxic atmosphere (nitrogen) 20-40 times as long as adults (unpublished experiments). Therefore the metabolic rate may be a major determinant of survival time, but other factors may also contribute.

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12. Karat, H. The greater resistance of very young animals to


