Effects of Phenobarbital in Cerebral Ischemia

Part II: Restitution of Cerebral Energy State, as well as of Glycolytic Metabolites, Citric Acid Cycle Intermediates and Associated Amino Acids After Pronounced Incomplete Ischemia

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SUMMARY Recovery of cerebral energy metabolism, following 15 or 30 min of pronounced, incomplete ischemia, was studied after 90 min of recirculation in rats that were either anesthetized with 70% N2O or 150 mg \( \cdot \) kg\(^{-1} \) of phenobarbital. In all animals arterial blood pressure, \( \text{Po}_2 \) and \( \text{PCO}_2 \) were close to normal during recirculation. In nitrous oxide-anesthetized animals kept ischemic for 30 min, but not in those given phenobarbital, a gradual rise in intracranial cerebrospinal fluid (CSF) pressure (to about 20–25 mm Hg) occurred during the last 20–30 min of recirculation. Following 15 min of ischemia, all phenobarbital-anesthetized animals, and 20 out of 24 animals anesthetized with 70% N2O, showed extensive restitution of cerebral energy metabolism, including normalization of phosphocreatine concentration, return of adenylate energy charge to about 99% of control, and disappearance of virtually all of the lactate accumulated during ischemia. These changes, and the pattern of changes in glycolytic and citric acid cycle intermediates, indicated that a near-normal mitochondrial metabolism returned. Following 30 min of ischemia in phenobarbital-anesthetized animals, a similar degree of recovery was observed. However, no animal maintained on 70% N2O showed such signs of metabolic recovery. The present results, and those previously reported from this laboratory, demonstrate that complete ischemia is followed by a significantly better recovery of cerebral energy metabolism than is a corresponding period of incomplete ischemia. Furthermore, the results demonstrate that phenobarbital protects under conditions of incomplete ischemia even when it does not prevent energy depletion from rapidly occurring during ischemia.

THIS SERIES of experiments was designed to study cerebral metabolic changes during incomplete ischemia, as well as in the recovery period following recirculation, and to assess the modulating influence of phenobarbital anesthesia. This second communication deals with restitution of brain energy metabolism following 15 and 30 min of severe, incomplete ischemia. It will be shown that although none of the superficially anesthetized animals (70% N2O) recovered a normal cerebral metabolic state following 30 min of ischemia, all animals given phenobarbital (150 mg \( \cdot \) kg\(^{-1} \) i.p.) demonstrated a near-complete normalization of the energy state. The possible explanations for the protective effects of barbiturate anesthesia in the present model of transient cerebral ischemia will be discussed. Furthermore, we will discuss possible biochemical mechanisms leading to irreversible neuronal damage, taking into account that a 30 min period of complete ischemia appears to be better tolerated than a similar period of incomplete ischemia.

Materials and Methods

Most of the experimental and analytical techniques used presently were described in the previous communication.\(^1\) In general, fed male Wistar rats
(250–350 g) were kept artificially ventilated on either 70% N₂O and 30% O₂ or 70% N₂ and 30% O₂, the latter animals being anesthetized with 150 mg·kg⁻¹ of phenobarbital. All animals were normothermic and normocapnic, and arterial P₀₂ was adjusted to at least 100 mm Hg. All animals were given tubocurarine (1 mg·kg⁻¹ i.v.) and heparin (100 I.U. i.v.). EEG and intracranial cerebrospinal fluid (CSF) pressure were continuously recorded. Both femoral arteries and veins were cannulated. The common carotid arteries were dissected free for later occlusion with rubber-coated clamps. Preparations were made for in situ freezing of cerebral cortical tissue.

After the operative procedures had been completed the animals were left undisturbed for 15–20 min. Cerebral ischemia was then induced for either 15 or 30 min, using a combination of bilateral carotid artery occlusion and reduction in blood pressure to 50 mm Hg (see Nordström and Siesjo's work). After cerebral ischemia had been maintained for 15 or 30 min, recirculation was started by removal of the arterial clamps and, simultaneously, by increasing blood pressure through reinfusion of the previously extracted blood. If necessary, the ventilation volume was adjusted and small amounts of sodium bicarbonate (1–2 ml of an isotonic solution) were given i.v. to compensate for occasional increases in Pco₂ and reductions in pH, respectively. Following recirculation for 90 min the brains were frozen in situ for subsequent metabolic analyses.

Analytical techniques, calculations and statistical methods were as described in the previous communication.

Results

Physiological Parameters

Changes in physiological parameters during the period of ischemia were similar to those described in the previous communication. Thus, a fall in arterial Pco₂ and a decrease in pH were seen, and these changes were usually more pronounced in animals anesthetized with nitrous oxide. When recirculation was started, adjustment of the ventilation volume and i.v. injection of sodium bicarbonate rapidly corrected these changes. The physiological parameters (arterial blood pressure, intracranial CSF pressure, body temperature, arterial P₀₂, Pco₂ and pH), as measured at the end of the 90 min recirculation period, are given in table 1 together with measured glucose, lactate and pyruvate concentrations. As the data show, arterial blood pressure and body temperature, as well as arterial Pco₂ and P₀₂, were close to control values. There was some persisting plasma acidosis that was somewhat more pronounced in animals anesthetized with nitrous oxide. Blood glucose concentrations were high (the animals were fed) and there were significant increases in lactate and pyruvate concentrations in animals anesthetized with 70% N₂O (30 min of ischemia).

When recirculation is started, a rapid normalization of cerebral perfusion pressure is necessary for restitution. The time course of the changes in arterial blood pressure and intracranial pressure (ICP) of the animals kept ischemic for 30 min is given in figure 1. As previously observed (e.g. Nilsson and Siesjo), barbiturate anesthesia in the rat results in a moderate decrease in arterial blood pressure. During the period of ischemia the blood pressure was identical (50 mm Hg), irrespective of the type of anesthesia. When recirculation was started, the increase in blood pressure was usually somewhat faster in the nitrous oxide group. However, both groups of animals reached normal blood pressure within 2 min. During the following recirculation period arterial blood pressure was kept above 120 mm Hg. When the recirculation was started the ICP rapidly increased and usually reached supranormal values during the first minutes (fig. 1). After about 10 min of recirculation ICP returned close to normal in both groups. During the late recirculation period a marked difference between the 2 groups of animals was observed. Thus, all animals anesthetized with phenobarbital showed a continuous normalization during the 90 min of recir-
culation, while a progressive increase in ICP was observed in all animals anesthetized with nitrous oxide.

EEG
Following occlusion of the common carotid arteries, the EEG remained unchanged as long as the arterial blood pressure was kept above 140-160 mm Hg. In the previous communication \(^1\) we reported that in some animals anesthetized with phenobarbital, a persisting low voltage, slow activity was recorded during the period of ischemia. In animals included in the present series of experiments, spontaneous EEG activity disappeared in all animals before the blood pressure reached 50 mm Hg. During the period of ischemia, no EEG activity was observed.

Following recirculation all animals anesthetized with phenobarbital recovered some spontaneous electrical activity. After 15 min of ischemia, the first signs of EEG activity appeared within 30 min of recirculation. In animals kept ischemic for 30 min, the first spontaneous electrical activity was seen after 30-60 min of recirculation. The first signs of EEG recovery appeared as intermittent bursts separated by isoelectric periods. During the recirculation period, the bursts became more and more frequent and, in some animals, a continuous high amplitude, slow activity was observed. However, no animals showed normalization of the EEG pattern during the period of recirculation. In nitrous oxide-anesthetized animals kept ischemic for 15 min all except 3 regained some spontaneous activity within 30 min, resembling that observed in animals anesthetized with phenobarbital. The 3 exceptional animals remained isoelectric during the 90 min period of recirculation. All nitrous oxide-anesthetized animals kept ischemic for 30 min remained isoelectric during the whole period of recirculation.

Biochemical Recovery
15 Min of Ischemia. Table 2 gives the concentrations of phosphocreatine (PCr), ATP, ADP, AMP and lactate, as well as the calculated values for adenylate energy charge (E.C.), sum of adenine nucleotides (2Ad) and lactate/pyruvate ratio. The phenobarbital-

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control (n = 17)</th>
<th>Phenoarb. (n = 6)</th>
<th>NO (n = 21)</th>
<th>NO recovery (n = 4)</th>
<th>Phenobarb. recovery (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCr</td>
<td>4.52 ± 0.06</td>
<td>4.98</td>
<td>3.51 ± 0.64**</td>
<td>5.25 ± 0.05***</td>
<td>5.63 ± 0.14</td>
</tr>
<tr>
<td>ATP</td>
<td>2.94 ± 0.03</td>
<td>3.06</td>
<td>1.68 ± 0.22***</td>
<td>2.60 ± 0.04***</td>
<td>2.63 ± 0.04</td>
</tr>
<tr>
<td>ADP</td>
<td>0.278 ± 0.004</td>
<td>0.276</td>
<td>0.398 ± 0.038***</td>
<td>0.291 ± 0.004*</td>
<td>0.269 ± 0.017</td>
</tr>
<tr>
<td>AMP</td>
<td>0.039 ± 0.002</td>
<td>0.045</td>
<td>0.218 ± 0.094***</td>
<td>0.030 ± 0.001</td>
<td>0.049 ± 0.008</td>
</tr>
<tr>
<td>σ· Ad</td>
<td>3.26 ± 0.02</td>
<td>3.39</td>
<td>2.29 ± 0.13***</td>
<td>2.93 ± 0.03***</td>
<td>2.96 ± 0.03</td>
</tr>
<tr>
<td>E.C.</td>
<td>0.945 ± 0.001</td>
<td>0.946</td>
<td>0.805 ± 0.060***</td>
<td>0.937 ± 0.001***</td>
<td>0.935 ± 0.005</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.88 ± 0.10</td>
<td>0.87</td>
<td>19.01 ± 6.10***</td>
<td>2.53 ± 1.8***</td>
<td>1.77 ± 0.37</td>
</tr>
<tr>
<td>Lactate/Pyruvate</td>
<td>15.4 ± 0.5</td>
<td>12.1</td>
<td>51.7 ± 15.4***</td>
<td>18.1 ± 2.8*</td>
<td>14.8 ± 1.3</td>
</tr>
</tbody>
</table>

*In a previous series of experiments, animals anesthetized with 70% NO and 150 mg·kg⁻¹ of phenobarbital (3 hr) were analyzed for cerebral metabolites. The results obtained allowed calculation of percentage change of each variable due to phenobarbital anesthetization. These percentage changes were applied to the present control values (70% NO) to obtain appropriate phenobarbital controls. Phenobarbital data were not treated statistically.
anesthetized animals recovered the energy charge to about 99% of control value. The level of PCr was above normal and the AMP and ADP concentrations were close to normal. The ATP concentration and the sum of the adenine nucleotides remained significantly below control. Twenty-one out of 24 animals anesthetized with nitrous oxide exhibited a similar degree of recovery of energy metabolites. However, in the nitrous oxide group, 3 animals remained isoelectric during the recirculation period. In these, a comparable recovery of tissue concentrations of organic phosphates was not obtained, and there was a pronounced lactic acidosis. In animals showing signs of extensive recovery, whether anesthetized with nitrous oxide or phenobarbital, tissue lactate concentrations were slightly higher than in the control situation. Since there was some increase in blood lactate (and pyruvate) concentrations the elevated tissue levels could have been partly due to equilibration between blood and cerebral extracellular fluids.

Results on glycolytic metabolites, citric acid cycle intermediates, associated amino acids, and ammonia are given in table 3. Animals under 70% N₂O that recovered some EEG activity (and cerebral energy state, see table 2) showed considerable recovery of glycolen concentration, increases in concentrations of glucose, G-6-P and F-6-P, and reductions in concentrations of FDP, DHAP and 3-PG, while pyruvate concentration was close to control. None of the citric acid cycle intermediates differed from control values. Changes in amino acids included reductions in glutamate and aspartate concentrations, and increases in glutamine and alanine concentrations, while values for GABA, ammonia and the sum of amino acids were close to control. A different pattern was observed in those animals that did not recover spontaneous EEG activity (or cerebral energy state, see table 2). Thus, there was marked accumulation of FDP, DHAP, 3-PG and pyruvate, increases in concentrations of all citric acid cycle intermediates except that of OAA (which was reduced), exaggerated changes in glutamate, aspartate, glutamine, and alanine, elevated ammonia levels, and a fall in the sum of amino acids.

### Table 3 Glycolytic Metabolites, Citric Acid Cycle Intermediates Associated Amino Acids and Ammonia in Control Animals (70% N₂O) and After 90 min Recirculation, Following 15 min of Ischemia in Animals Anesthetized with 70% N₂O or Phenobarbital (150 mg·kg⁻¹). The Values are Means ± SEM

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control N₂O (n = 17)</th>
<th>N₂O No EEG recovery (n = 3)</th>
<th>N₂O EEG recovery (n = 9)</th>
<th>Phenobarbital EEG recovery (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen</td>
<td>2.38 ± 0.09</td>
<td>0.59 ± 0.17***</td>
<td>1.85 ± 0.17**</td>
<td>2.90 ± 0.20</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.27 ± 0.24</td>
<td>9.89 ± 0.67***</td>
<td>9.70 ± 1.21***</td>
<td>9.06 ± 1.01</td>
</tr>
<tr>
<td>G-6-P</td>
<td>0.10 ± 0.004</td>
<td>0.272 ± 0.005***</td>
<td>0.175 ± 0.003***</td>
<td>0.156 ± 0.012</td>
</tr>
<tr>
<td>F-6-P</td>
<td>0.015 ± 0.001</td>
<td>0.030 ± 0.003***</td>
<td>0.021 ± 0.001***</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>F-D-P</td>
<td>0.084 ± 0.004</td>
<td>0.250 ± 0.061***</td>
<td>0.062 ± 0.008***</td>
<td>0.067 ± 0.010</td>
</tr>
<tr>
<td>DHAP</td>
<td>0.024 ± 0.001</td>
<td>0.103 ± 0.031***</td>
<td>0.017 ± 0.001***</td>
<td>0.015 ± 0.002</td>
</tr>
<tr>
<td>3-PG</td>
<td>0.037 ± 0.003</td>
<td>0.117 ± 0.030***</td>
<td>0.024 ± 0.005**</td>
<td>0.024 ± 0.005</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>0.119 ± 0.004</td>
<td>0.358 ± 0.019***</td>
<td>0.144 ± 0.018</td>
<td>0.120 ± 0.025</td>
</tr>
<tr>
<td>Citrate</td>
<td>0.324 ± 0.009</td>
<td>0.478 ± 0.036***</td>
<td>0.337 ± 0.017</td>
<td>0.344 ± 0.029</td>
</tr>
<tr>
<td>α-keto-glutarate</td>
<td>0.160 ± 0.006</td>
<td>0.203 ± 0.036*</td>
<td>0.142 ± 0.002</td>
<td>0.127 ± 0.016</td>
</tr>
<tr>
<td>Fumarate</td>
<td>0.068 ± 0.004</td>
<td>0.100 ± 0.007***</td>
<td>0.072 ± 0.005</td>
<td>0.057 ± 0.004</td>
</tr>
<tr>
<td>Malate</td>
<td>0.411 ± 0.024</td>
<td>0.625 ± 0.045**</td>
<td>0.349 ± 0.022</td>
<td>0.348 ± 0.046</td>
</tr>
<tr>
<td>Oxaloacetate</td>
<td>6.5 X 10⁻³</td>
<td>4.4 X 10⁻³**</td>
<td>5.7 X 10⁻³</td>
<td>6.2 X 10⁻³</td>
</tr>
<tr>
<td>Glutamate</td>
<td>12.80 ± 0.14</td>
<td>8.16 ± 0.33**</td>
<td>9.93 ± 0.27***</td>
<td>10.11 ± 0.30</td>
</tr>
<tr>
<td>Aspartate</td>
<td>3.52 ± 0.05</td>
<td>1.17 ± 0.15**</td>
<td>2.92 ± 0.18**</td>
<td>3.40 ± 0.15</td>
</tr>
<tr>
<td>Glutamine</td>
<td>5.91 ± 0.18</td>
<td>9.22 ± 0.63***</td>
<td>8.60 ± 0.32***</td>
<td>8.26 ± 0.37</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.534 ± 0.018</td>
<td>2.020 ± 0.270***</td>
<td>0.825 ± 0.076**</td>
<td>0.765 ± 0.116</td>
</tr>
<tr>
<td>GABA</td>
<td>2.33 ± 0.05</td>
<td>2.81 ± 0.29***</td>
<td>2.14 ± 0.28</td>
<td>1.70 ± 0.05</td>
</tr>
<tr>
<td>NH₄⁺</td>
<td>0.277 ± 0.010</td>
<td>0.519 ± 0.107***</td>
<td>0.295 ± 0.020</td>
<td>0.266 ± 0.036</td>
</tr>
<tr>
<td>Σ Amino acids</td>
<td>24.93 ± 0.25</td>
<td>23.37 ± 0.61*</td>
<td>24.41 ± 0.23</td>
<td>24.22 ± 0.52</td>
</tr>
</tbody>
</table>

*Since control animals were maintained on 70% N₂O, the phenobarbital data were not treated statistically.
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<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>No 70% N2O (n = 12)</th>
<th>Phenobarb. (n = 6)</th>
<th>No EEG recovery</th>
<th>Phenobarb. EEG recovery (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>4.48 ± 0.06</td>
<td>4.98</td>
<td>2.54 ± 0.50***</td>
<td>5.61 ± 0.11</td>
<td></td>
</tr>
<tr>
<td>ATP</td>
<td>2.98 ± 0.01</td>
<td>3.06</td>
<td>1.14 ± 0.20***</td>
<td>2.61 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>ADP</td>
<td>0.277 ± 0.006</td>
<td>0.276</td>
<td>0.370 ± 0.024**</td>
<td>0.266 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>AMP</td>
<td>0.039 ± 0.002</td>
<td>0.045</td>
<td>0.360 ± 0.040**</td>
<td>0.046 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Σ Ad</td>
<td>3.31 ± 0.02</td>
<td>3.39</td>
<td>1.87 ± 0.16</td>
<td>2.92 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>E.C.</td>
<td>0.946 ± 0.001</td>
<td>0.946</td>
<td>0.663 ± 0.056***</td>
<td>0.938 ± 0.003</td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>1.92 ± 0.20</td>
<td>0.87</td>
<td>28.9 ± 3.9***</td>
<td>2.16 ± 0.38</td>
<td></td>
</tr>
<tr>
<td>Lact/Pyr</td>
<td>15.0 ± 1.3</td>
<td>12.1</td>
<td>108 ± 27**</td>
<td>12.8 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Concentrations of PCr, ATP, ADP, AMP and Lactate, as Well as Calculated Values for the Sum of Adenonucleotides (Σ Ad), Energy Charge (E.C.), and the Lactate/Pyruvate Ratio after 90 min of Recirculation Following 30 min of Ischemia in Animals Anesthetized With Nitrous Oxide or Phenobarbital. The Values are Means ± SEM.

Table 5: Concentrations of Glycolytic Metabolites, Citric Acid Cycle Intermediates and Associated Amino Acids, as Well as Ammonia After 90 min of Recirculation Following 30 min of Ischemia in Animals Anesthetized With Nitrous Oxide or Phenobarbital. The Values are Means ± SEM in μmol·g⁻¹ of Wet Tissue.

Since the control animals were maintained on 70% N2O, the phenobarbital data were not treated statistically.

After 30 min of complete ischemia, the ATP concentration and the sum of the adenine nucleotides remained significantly below control.

Table 5 shows changes in the concentrations of glycolytic metabolites, citric acid cycle intermediates, associated amino acids, and ammonia. In animals maintained on 70% N2O the pattern of changes in glycolytic metabolites and citric acid cycle intermediates was very similar to that observed after 15 min of ischemia in animals showing no EEG recovery (see table 3). Since control animals were maintained on 70% N2O, the results pertaining to phenobarbital anesthesia in tables 3 and 5 do not illustrate changes from appropriate control values. In order to allow such a comparison, values for glycolytic metabolites, citric acid cycle intermediates, amino acids, and ammonia have been given in percent of control in figure 2. (Control values were derived as described in the
FIGURE 2. Changes in glycolytic metabolites, citric acid cycle intermediates, associated amino acids, and ammonia after 90 min of recirculation, following 15 or 30 min of ischemia in animals anesthetized with phenobarbital (150 mg·kg⁻¹). The values are given as per cent of phenobarbital control using conversion factors derived from Chapman et al. * (see Table 2).

Discussion

We will discuss in turn the pattern of metabolic changes obtained in the present experiments, the differences in metabolic restitution following complete and incomplete cerebral ischemia, possible protective effects of barbiturates in the present model of transient cerebral ischemia, and biochemical mechanisms for production of irreversible neuronal damage. The latter part of the Discussion is an attempt to synthesize results obtained in several previous communications.1, 4, 8-10

Metabolic Recovery Following Incomplete Ischemia

Following 15 min of pronounced, incomplete cerebral ischemia all animals (except 3 in the nitrous oxide group) exhibited a similar pattern of recovery of cerebral metabolism. We will, therefore, concentrate on results obtained after 30 min of ischemia. In all animals anesthetized with phenobarbital (150 mg·kg⁻¹) recovery of cerebral energy state was near-complete. The concentrations of the metabolites of the energy reserve were almost identical to those seen after 30 min of complete cerebral ischemia in animals anesthetized with either nitrous oxide or phenobarbital.

Typically, the recovery observed involved complete restoration of normal (or supernormal) concentrations of PCr and glycogen, normalization of ADP and AMP concentrations, return of the adenylate energy charge to about 99% of control, disappearance of most of the lactate accumulated during ischemia, signs of phosphofructokinase inhibition, and extensive normalization of tissue concentrations of citric acid cycle intermediates, associated amino acids, and ammonia. However, apart from the fact that the energy charge does not completely normalize, there is a lingering reduction in ATP concentration and in the sum of adenine nucleotides.

There are two possible explanations for the postischemic reduction in ATP concentration.11-12 First, the reduction may reflect slow resynthesis of adenine nucleotides. Ischemia leads to a decrease in the sum of adenine nucleotides,9, 13-15 the mechanisms involving deamination and dephosphorylation of AMP,16-19 with possible loss of degradation products to extracellular fluid. Since both de novo synthesis of adenine nucleotides and transport of purine bases or nucleosides between blood and brain are slow processes,18, 20 it may take much longer than 90 min to achieve resynthesis of adenine nucleotides.12 Second, it is possible that the persisting reduction in nucleotide pool size reflects irreversible neuronal damage. This suggestion is supported by the finding that resynthesis of ATP was not observed during a 24 hr recovery period,11 following transient ischemia in the gerbil. It seems plausible that if cell death occurs, there is eventually loss of all adenine nucleotides. Thus, normalization of the adenylate energy charge does not exclude cell death.

An increase in PCr concentration following tran-
sient cerebral ischemia has frequently been observed. The explanation for this increase is not clear, but it has tentatively been attributed to either an increase in intracellular pH or a change in the apparent equilibrium constant for the creatine kinase reaction. Thus, normalization of the PCR concentration does not necessarily indicate restitution of cerebral energy state.

There is no histopathological evaluation of cell damage with the present model of ischemia. Following complete ischemia of 15 min duration, there is histopathological damage to only a small proportion of cells. In these animals, the pattern of recovery of cerebral energy metabolism is very similar to that observed in the present phenobarbital-anesthetized animals (see Ljunggren et al. On the basis of these facts, we tentatively conclude that cell damage may have been relatively slight.

In contrast to phenobarbital-anesthetized animals, no animals anesthetized with nitrous oxide showed a comparable restitution of energy state following 30 min of ischemia. Since a progressive increase in ICP was seen during the last 30-40 min of recirculation in the latter animals, it is probable that the brain damage was irreversible and that total brain infarction would eventually have developed. It seems less likely that the protective effect observed in animals anesthetized with phenobarbital can be attributed to a less pronounced perturbation of energy metabolism during the period of ischemia. Thus, all animals in the present series were isoelectric during the period of ischemia irrespective of the type of anesthesia. In the previous communication it was shown that the derangement of the energy metabolism was identical in all isoelectric animals. Presently, it cannot be judged whether metabolic damage solely results from events occurring during ischemia, or if postischemic factors come into play. The delayed rise in ICP favors the latter possibility. Theoretically, phenobarbital could act by reducing blood flow and intracranial volume, thereby preventing increase in ICP and circulation failure. However, since postischemic CBF values are similar in animals anesthetized with nitrous oxide and phenobarbital, it seems more likely that metabolic events occurring in the recirculation period lead to brain swelling and that circulatory failure is a secondary phenomenon.

The present results on carbohydrate intermediates and amino acids give additional information on recovery of mitochondrial metabolism. In phenobarbital-anesthetized animals the pattern of changes in glycolytic metabolites and citric acid cycle intermediates is similar to that described following 5 or 30 min of complete cerebral ischemia in superficially anesthetized animals. Most importantly, there were increases in the concentrations of G-6-P and F-6-P, and a reduction in FDP concentration. If it is assumed that oxidation of (accumulated) lactate to pyruvate occurs part of the postischemic substrate requirements, and that glycolytic flux is decreased, the pattern of changes in glycolytic intermediates is compatible with inhibition of phosphofructokinase. In animals which failed to recover (nitrous oxide group) the concentrations of glucose and of all glycolytic metabolites measured were markedly increased. Presumably, these alterations reflect lack of control of glycolysis (absence of Pasteur effect), as would be expected in a situation with reduced concentrations of PCR and ATP, and increased concentrations of ADP, AMP and ammonia.

Previous results from the laboratory have shown that after 15 min of recirculation, following 5 min of ischemia, tissue concentrations of glutamate and aspartate are reduced while those of glutamine, GABA and alanine are increased. The present experiments show that, after prolonged recirculation, alanine has a tendency to decrease toward control levels, and GABA levels normalize. This is in accordance with observations by others. Nitrous oxide-anesthetized animals, that failed to restitute energy metabolism, showed a pronounced perturbation of amino acid concentrations, a decrease in the sum of amino acids, and accumulation of ammonia. Possibly, these changes reflect a deranged mitochondrial metabolism.

Recovery Following Complete and Incomplete Ischemia

Previous results from this laboratory, and the present ones, demonstrate that 1) irrespective of the depth of anesthesia all animals show extensive restitution of cerebral energy metabolism after 30 min of complete cerebral ischemia, 2) following the same period of incomplete ischemia, no animal under 70% N2O shows a corresponding normalization of cerebral metabolic state, and 3) under conditions of incomplete ischemia, phenobarbital anesthesia has a dramatic protective effect. It was suggested by Hossmann and Kleihues that recovery was adversely affected if the ischemia was incomplete. This observation has been disputed, but is in accordance with our results. This seemingly paradoxical finding is perhaps not totally unexpected since a trickling blood flow during the ischemic period creates a more complex situation from a microcirculatory as well as from a biochemical point of view.

Deficient recirculation has been suggested as a factor limiting restitution following transient cerebral ischemia. Insufficient microcirculation may be caused by a combination of pathophysiological mechanisms such as swelling of endothelial and perivascular cells, intravascular aggregation of blood corpuscles, and increased blood viscosity. Although it is well established that ischemic brain damage may occur also in the absence of a primary circulatory insufficiency, it is reasonable to assume that microvascular impairment may be of importance in some experimental and clinical conditions. It should be added that the "no-reflow" phenomenon does not seem to be restricted to the brain, and in vivo studies have shown that the sludge phenomenon may occur in skeletal muscle during experimental shock. Thus, the possibility must be considered that a trickling blood flow during hypotension, as in the present experimen-
tal situation, results in an inhomogeneous microcirculation postischemically. However, if this is correct, it is difficult to explain the protection afforded by barbiturates, especially since blood flow measurements revealed no consistent differences between the 2 groups of animals. Thus, we must also consider the possibility that incomplete ischemia is primarily more deleterious for cellular metabolism.

Mechanisms of Cellular Damage

From light and electron microscopic studies it has been shown that the first signs of cellular damage, leading to autolysis, affect the mitochondria and the endoplasmatic reticulum in neurons as well as in other cells. In this context, it should be mentioned that, following repeated short periods of cerebral ischemia, signs of mitochondrial uncoupling have been observed.

Little is known about the biochemical alterations that underlie cellular autolysis. It has been assumed by many that pronounced tissue acidosis, e.g., by causing release or activation of lysosomal enzymes, can cause irreversible cell damage. Although it was shown by Ljunggren et al. that pronounced cellular acidosis during a short period (5 min) of complete ischemia is compatible with recovery of cerebral energy metabolism, the mechanism may still be of importance in situations with longer periods of ischemia. In support of this hypothesis, it has recently been reported that histological damage is more pronounced in animals made hyperglycemic prior to a period of complete cerebral ischemia. In the present experimental model, the persisting blood flow leads to extreme degrees of tissue lactacidosis. However, since animals anesthetized with phenobarbital develop a similar degree of lactate accumulation, the present results leave no direct support to the view that irreversible cell damage is triggered by lactic acid accumulation.

Two observations indicate that the final damage resulting from transient ischemia may, at least partly, be incurred in the recirculation period. First, Bleyaert et al. found that thionipental ameliorates brain damage following complete ischemia even when given 5 min after the start of recirculation. Second, Cooper et al. have reported that cerebral ribosomes are intact during complete ischemia but rapidly dis-aggregate and lose their ability to initiate polypeptide formation upon recirculation. It is also of importance that phenobarbital protected against metabolic damage in the present model although it did not prevent energy failure from occurring during ischemia.

There is little information on possible mechanisms that could aggravate ischemic cell damage during the period of reoxygenation. Recently, it has been suggested that ischemic damage could result from formation of free (oxygen) radicals. Free radical reactions have previously been proposed as an explanation for cell injury in liver and other tissues. Recently, Demopoulos et al. suggested that such mechanisms may lead to peroxidative membrane damage in regional ischemia due to MCA ligation. A difficulty with this hypothesis is that the rate of free radical formation is proportional to oxygen tension. However, if it is assumed that damage occurs during recirculation, when oxygen utilization is low and CBF may be increased, free radical damage does not seem unlikely. Presently, the only suggestive evidence is that barbiturates seem to act as free radical scavengers.

Whatever is the correct explanation of the differences in recovery following complete and incomplete ischemia, and of the effects of barbiturates it must be seriously considered that part of the cell damage occurring after transient cerebral ischemia may be due to oxygen-dependent mechanisms.

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