Recovery of Brain Mitochondrial Function in the Rat after Complete and Incomplete Cerebral Ischemia

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SUMMARY Respiratory function was evaluated in brain mitochondria prepared from rats subjected to either complete compression ischemia or pronounced incomplete hypotensive ischemia of 30 min duration, and from animals allowed a 30 min recirculation period following 30 min of ischemia. Oxygen utilization rates in the mitochondrial preparations were measured with an oxygen electrode in a closed and stirred chamber with glutamate plus malate or with succinate as substrates.

After 30 min of ischemia there was a decrease in respiratory control ratio (RCR), in state 3 respiratory activity and maximal phosphorylation rate whether ischemia was complete or incomplete. After recirculation following complete ischemia, mitochondria showed extensive functional recovery with normalization of RCR, as well as of state 3 and maximal phosphorylation rates. Following incomplete ischemia, there was a suggestive further deterioration of mitochondrial function. Addition of Mg$^{2+}$ did not reverse the pattern of respiratory inhibition. The results are in agreement with previous communications from this laboratory, demonstrating a nearly complete recovery of cerebral energy state upon recirculation after an equivalent period of complete compression ischemia but not after pronounced, incomplete hypotensive ischemia. The persistence of mitochondrial dysfunction during recirculation after incomplete ischemia indicates that a mitochondrial damage could be a primary factor for the deficient recovery of the cerebral energy state. Events during the initial recirculation period may be at least partly responsible for failure of energy metabolism.

AS A RESULT OF experimental work during the last few years, earlier opinions concerning the sensitivity of cerebral tissue to anoxia and ischemia have changed. Thus, although integrated brain function does not return, extensive recovery of neurophysiological parameters, such as spontaneous electrocortical activity and evoked response, as well as of cerebral energy metabolism, has been shown to be possible even after periods of complete cerebral ischemia of 60 min duration in the cat and monkey. In these experiments barbiturate anesthesia was used. In lightly anesthetized (70% N₂O) rats a virtually complete recovery of cerebral energy state was found during recirculation after a 30 min period of complete compression ischemia. However, no comparable recovery occurred during recirculation after a similar period of severe, incomplete hypotensive ischemia. In the latter type of ischemia, protection was afforded by barbiturate anesthesia. These results indicate that transient incomplete ischemia is more deleterious for brain tissue than complete cessation of cerebral blood flow. The pathophysiological mechanisms responsible for this difference are poorly understood. Histopathological investigations have demonstrated that mitochondrial alterations are the first sign of cell damage in brain anoxia-ischemia. However, mitochondrial function, as evaluated from the respiratory activity of isolated brain mitochondria, has been found relatively resistant to ischemia. There is no information about the recovery of mitochondrial respiratory function upon recirculation following ischemic periods known to cause significant depression of respiratory rates.

The present investigation was undertaken to evaluate mitochondrial function in brain tissue after a 30 min period of complete and incomplete ischemia, as well as during recirculation.

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Materials and Methods

Operative and Sampling Techniques

All experiments were performed on adult male Wistar rats (350–400 g) which had free access to water and rat pellets until operation. The animals were given analgesia with 80% N₂O and 20% O₂. Surgical regions were locally infiltrated with lidocaine (10 mg/ml), 0.4–0.5 ml per animal. The rats were then paralyzed with 1.5 mg tubocurarine i.p. and, following tracheotomy, artificially ventilated, using a Starling type of respirator, with 70% N₂O and 30% O₂. Both femoral arteries and one femoral vein were cannulated for continuous blood pressure recording, blood withdrawal and, in some of the experiments, for infusion of blood. All animals were heparinized (100 I.U. i.v.). After the operation, the animals were allowed a steady state period of 20–30 min before the experiment was started.

Arterial oxygen tension (Pao₂), carbon dioxide tension (Paco₂), as well as pH, were measured using microelectrodes operated at 37°C, with due corrections for body temperature. Ventilation was adjusted to give a Pao₂ of about 100 mm Hg and Paco₂ of 35–40 mm Hg. Body temperature was kept close to 37°C and the brain temperature was prevented from falling by a heating bulb, placed at a predetermined distance from the head.

Controls (n = 6). The operative procedure was the same as in the ischemic groups. The animals were kept artificially ventilated with 70% N₂O + 30% O₂ for one hour. They were then decapitated and brain mitochondria isolated as described below.

Complete Ischemia (n = 8). Ischemia was induced as described by Ljunggren et al.¹³ and Nordström et al.⁶ The atlanto-occipital membrane was exposed and a double-barrelled cannula inserted in the cisterna magna. Intracranial hypertension was produced by infusion of artificial CSF (kept at 37°C), momentarily increasing intracranial pressure (ICP) to 50–70 mm Hg above the systolic blood pressure. A deleterious rise in blood pressure was prevented by i.v. infusion of Arfonad® (trimethaphan-camphor-sulfonate). After the initial 5–10 min period, the infusion rate of CSF was regulated to obtain an ICP level 20–30 mm Hg above the systolic blood pressure. At the end of the 30 min period of ischemia, 4 animals were decapitated for isolation of mitochondria. In 4 animals the brains were recirculated by stopping the infusion of artificial CSF for 30 min before decapitation.

Incomplete Cerebral Ischemia (n = 9). Incomplete ischemia was induced by bilateral clamping of the carotid arteries combined with arterial hypotension (by bleeding) as described by Nordström et al.⁵,⁷ Mean arterial blood pressure was kept constant at 50 mm Hg by means of an automatically controlled syringe connected to one of the femoral arteries and one vein, extracting or reinfusing blood whenever needed. Four of the animals were decapitated after 30 min of ischemia. In 5 of the animals the brains were recirculated for 30 min by removing the arterial clamps and reinfusing the blood. To counteract excessive systemic acidosis, caused by the arterial hypotension, 0.5–1.0 cc of 0.6 M sodium bicarbonate was infused i.v. during the ischemic period and 0.5 cc given i.v. at the beginning of the recirculation period. The EEG was continuously recorded in all animals subjected to incomplete ischemia, using gold-plated copper screws inserted into the skull bone in the frontotemporal regions.

Isolation of Mitochondria

At the end of the experimental period the animals were decapitated using a guillotine, the skull bone was opened, the cerebellum and the medulla oblongata were cut away, and the brain dropped into ice-cold isolation medium. This procedure was performed within 20 sec. The brain mitochondria were isolated by a modification of the techniques of Clark and Nicklás¹⁰ as described by Ginsburg et al.¹² Immediately after transferring the brain into the isolation medium (0.225 M mannitol, 0.075 M sucrose and 100 μM EGTA), it was quickly cut into small pieces and washed several times with the isolation medium. Ten ml of a mannitol-sucrose-EGTA solution containing the bacterial proteinase Nagarse (0.5 mg • ml⁻¹) and bovine serum albumin (BSA) (2.5 mg • ml⁻¹) were added. After gentle homogenization of the tissue in a hand-operated Dounce homogenizer, it was incubated with the proteinase solution for 60 sec. Fresh isolation medium with BSA (5 mg • ml⁻¹ of isolation medium) was then added, and the mixture was rehomogenized before centrifugation for 3 min at 2200 × g in a Sorvall centrifuge, operated at 0–5°C. The resulting pellet was resuspended and centrifuged at the same speed for another 3 min. The supernatant fractions containing mitochondria were spun for 8 min at 12100 × g and the new supernatant was discarded. The pellet was gently rehomogenized with a glass rod and suspended in 3% Ficoll solution and layered over a 6% Ficoll solution before centrifugation at 10400 × g for 30 min. After discarding the supernatant the pellet was resuspended in isolation medium and again spun for 10 min at 12100 × g to wash the mitochondrial fraction from Ficoll. The resulting pellet was rehomogenized and resuspended in 0.2 ml of isolation medium. Ten µl of the suspension were taken for protein assay. All solutions, as well as the mitochondrial suspension, were kept on ice during the isolation procedure, and until the analysis was started.

Respiratory Activity. Respiratory activity of the mitochondrial suspension was measured polarographically in a closed and magnetically stirred chamber with an oxygen electrode operated at room temperature (23°C). The reaction medium consisted of 0.225 M mannitol, 0.075 M sucrose, 1 mM EGTA, 0.01 M H₂PO₄, and 0.01 M HCl that was neutralized to pH 7.40 using Tris (hydroxymethyl) aminomethane (Trizma® Base, Sigma.) Twenty-five µl of the mitochondrial suspension were added to 0.625 ml of the reaction medium in the oxygen electrode chamber. State 4 respiratory activity was determined as the oxygen consumption rate with 7.5 mM glutamate plus...
7.5 mM malate as NAD-linked substrates and with 7.5 mM succinate as a FAD-linked substrate, after blocking the electron transfer from NAD to cytochrome b by 3 μM Rotenone. State 3 respiratory activity was measured as the O₂ consumption rate after the addition of substrates and ADP (460 μM with NAD-linked and 310 μM with FAD-linked substrates). When glutamate plus malate were used as substrates state 3 respiratory activity was measured in the presence of Mg²⁺ (1.5 mM MgCl₂) as well. The concentration of mitochondrial protein was assayed according to the method of Lowry et al.¹⁴

Cytochrome (a + a₃) was determined with a dual wavelength spectrophotometer (Aminco-Chance D-W 2) at 445-460 nm as the difference in optical density after the addition of substrates and ADP (460 nm) between the fully oxidized (after Rotenone addition) and the fully reduced state (after ADP addition using succinate as substrate and anaerobiosis).

**Calculations**

The concentration of cytochrome (a + a₃) was calculated as the measured difference in optical density using an extinction coefficient of 160 mM⁻¹ cm⁻¹. The oxygen consumption rates in state 3 (substrate + ADP stimulated) and in state 4 (only substrate) were calculated as mol of oxygen utilized per mol of cytochrome (a + a₃), or μmol O₂ per mg of mitochondrial protein per min. Respiratory control ratio (RCR) was calculated as state 3 divided by state 4 respiratory activities. ADP/O was calculated as the ratio between the added amount of ADP (μmol·l⁻¹) and the amount of oxygen consumed (expressed as μatoms·l⁻¹) as measured with the oxygen electrode with the different substrates (see above).

As the number of animals in each group was small (4–6) and normal distributed values cannot be assumed, non-parametric statistical methods were used. Most values are presented as means and ranges; the respiratory activities (expressed as mol O₂·mol (a + a₃)²⁺·min⁻¹) are given as individual values (figs. 2 and 3). However, to facilitate inspection of data some results are summarized in figures and tables as means ± sem. Differences between groups were evaluated statistically by using Wilcoxon’s rank sum test (two-tailed).

**Results**

In animals subjected to complete ischemia the mean arterial blood pressure fell to minimal values of 60 mm Hg during a short period (1–2 min) as a result of the infusion of Arfonadé® (see Methods), but remained stable around 100 mm Hg for the rest of the ischemic period. In all animals recirculated after incomplete ischemia the mean arterial blood pressure was brought back to at least 120 mm Hg within 2 minutes at the beginning of the recirculation period.

Physiological parameters are given in table 1. There were no differences in body temperature, mean arterial blood pressure (MABP), blood gases (Pao₂, Paco₂), or pH at the beginning of the experimental period. The decrease in arterial Paco₂ and pH during the period of incomplete ischemia has been observed earlier in this experimental model and is attributed to peripheral vasoconstriction due to the induced hypovolemia.¹⁶ During the recirculation period Paco₂ normalized and pH increased toward normal, but was still slightly below the control value.

In the groups subjected to incomplete ischemia, the EEG was continuously recorded. This was done to provide some measure of the severity of the ischemia, and to ascertain that the ischemia induced was similar in all experiments and comparable to earlier published material concerning recovery of cerebral energy state.⁷ In all animals spontaneous electrocortical activity was abolished during the ischemic period. In recirculated animals no sign of returning electrocortical activity was observed, except in one animal. In this animal continuous electrocortical activity (low voltage, slow wave pattern) returned as soon as after only 20 min of recirculation. As a similar early EEG recovery has never been observed in the same experimental situation (cf. Nordström et al. 1978⁷), we conclude that the degree of ischemia in this animal must have been different, possibly due to technical errors. For this

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**Table 1. Physiological Parameters in Control Animals, Animals Subjected to 30 min of Either Complete Compression Ischemia or Incomplete Hypotensive Ischemia as Well as After 30 min of Normotensive Recirculation. Values are Means ± SEM.**

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Body temp. (°C)</th>
<th>MABP (mm Hg)</th>
<th>Paco₂ (mm Hg)</th>
<th>Paco₃ (mm Hg)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>37.2 ± 0.2</td>
<td>152 ± 4</td>
<td>36.8 ± 1.2</td>
<td>104 ± 7</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>Complete ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before induction (n = 8)</td>
<td>37.3 ± 0.2</td>
<td>152 ± 7</td>
<td>38.9 ± 1.3</td>
<td>102 ± 4</td>
<td>7.40 ± 0.02</td>
</tr>
<tr>
<td>30 min ischemia¹ (n = 4)</td>
<td>37.2 ± 0.2</td>
<td>98 ± 13</td>
<td>34.9 ± 1.5</td>
<td>145 ± 15</td>
<td>7.44 ± 0.02</td>
</tr>
<tr>
<td>30 min recirc. (n = 4)</td>
<td>37.1 ± 0.1</td>
<td>138 ± 5</td>
<td>31.0 ± 1.0</td>
<td>134 ± 12</td>
<td>7.45 ± 0.04</td>
</tr>
<tr>
<td>Incomplete ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>before induction (n = 8)</td>
<td>37.1 ± 0.3</td>
<td>149 ± 3</td>
<td>37.8 ± 1.4</td>
<td>100 ± 4</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>30 min ischemia¹ (n = 4)</td>
<td>37.8 ± 0.3</td>
<td>50</td>
<td>26.4 ± 3.0</td>
<td>125 ± 14</td>
<td>7.15 ± 0.05</td>
</tr>
<tr>
<td>30 min recirc. (n = 4)</td>
<td>37.8 ± 0.2</td>
<td>131 ± 7</td>
<td>37.0 ± 2.5</td>
<td>140 ± 26</td>
<td>7.29 ± 0.03</td>
</tr>
</tbody>
</table>

¹Measured in separate animals, treated in the same way.
reason this animal was excluded from the group (and the statistical evaluations). We did, however, analyse the mitochondrial function in this animal. The data will be reported separately.

Mitochondrial Yield

The total protein and cytochrome (a + a₃) yield in the different experimental groups are given in table 2. A slight but significant decrease in mitochondrial protein yield was found after 30 min of complete ischemia but protein content did not differ significantly from the control value after 30 min of recirculation. In the incomplete ischemic model the decrease in mitochondrial protein (to about 70% of control) was more pronounced during the ischemic period and the protein yield was still significantly lower after recirculation than in the control group. The total amount of cytochrome (a + a₃) recovered was 1.8 nmol (mean; range 0.7-2.3) in the control group and the same amount was found during ischemia and upon recirculation in the animals subjected to complete ischemia. There were suggestive, but not significant, decreases during both incomplete ischemia (mean 1.3 nmol, range 0.9-1.7) and recirculation (mean 1.2 nmol, range 0.8-1.4).

Since there was some discrepancy between protein and cytochrome (a + a₃) yields, which could mean variable degrees of non-mitochondrial contamination (see Discussion) the oxygen consumption rates are given with both protein and cytochrome (a + a₃) concentrations as references.

Mitochondrial Respiratory Activity

Complete Ischemia and Recirculation. Examples of oxygen electrode measurements are given in fig. 1 (b, c) and compared to results obtained in control animals (fig. 1 a). During the 30 min period of complete cerebral ischemia the respiratory control ratio (RCR) decreased to about half the control value, with glutamate and malate as substrates, no significant decrease in RCR (mean 4.3). This was due to a slight decrease in state 4 activity concomitant with a reduction in state 3 respiratory activity. There was a suggestive decline in ADP/O ratio from 1.8 to 1.4 in ischemia which normalized during recirculation (table 3).

In order to explore the possibility that the observed deterioration in mitochondrial function during ischemia was not merely a result of increased intracranial pressure, rather than the ischemic situation per se, complete ischemia was induced in 3 additional experimental animals by decapitation. The heads were kept in a waterbath at 37°C for 30 minutes before isolation of the brain mitochondria. Table 4 shows that the individual values in these animals were in the same range as described for the model of reversible complete ischemia.

Incomplete Ischemia and Recirculation

Examples of oxygen electrode recordings are given in fig. 1 (d, e). After a 30 min period of incomplete ischemia the functional state of the brain mitochondria was similar to that observed in complete ischemia. When glutamate and malate were used as substrates, RCR decreased from a control value of 6.5 (mean) to 4.2 (table 3) with a corresponding decrease in state 3 respiratory activity (from control 417 to 270 mol O₂ • mol cyt. (a + a₃)⁻¹ • min⁻¹; see fig. 2 b). Upon recirculation, 4 animals out of 5 did not recover any spontaneous electrocortical activity. In these

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Total Yield of Brain Mitochondrial Protein and Cytochrome (a+aa) in Control Animals, in Animals Subjected to 30 min of Complete or Incomplete Cerebral Ischemia, as Well as in Those Studied After 30 min of Recirculation. Mean Values and Ranges (in Brackets).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total mitochondrial yield</td>
<td>Control (n = 8)</td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>7.3 (7.5-8.5)</td>
</tr>
<tr>
<td>Cytochrome (a + a₃) (nmol)</td>
<td>1.8 (0.7-2.3)</td>
</tr>
</tbody>
</table>

**p ≤ 0.01 indicates statistical significant differences from control.
animals brain mitochondrial respiratory function was still significantly impaired with RCR = 3.5, ADP/O = 2.2 (table 3) and state 3 respiratory activity = 199 mol O₂/mol cyt. (a + a₃)¹⁻¹·min⁻¹ (mean values) when glutamate plus malate were used as substrates (fig. 2 b). A further decrease of state 3 respiratory activity during recirculation in comparison to the ischemic situation was close to significance (p = 0.058). The pattern of changes in state 3 respiratory activity was not influenced by the addition of Mg²⁺, as this only caused small and similar increases in state 3 rates with the mitochondria isolated from control, ischemic or recirculated brains.

When succinate was used as substrate (fig. 3 b) a similar pattern of state 3 inhibition was found during ischemia and recirculation, while the suggestive decreases in RCR and ADP/O ratios during ischemia reached statistical significance during the recirculation period (table 3).

One animal was excluded from the group because of early EEG recovery, indicating a different ischemic level. In this animal the functional status of the isolated brain mitochondria was different: state 3 and state 4 respiratory activities were 570 and 97 mol O₂/mol cyt. (a + a₃)¹⁻¹·min⁻¹ with glutamate plus malate (Mg²⁺ not added), and 380 and 96 mol O₂/mol cyt. (a + a₃)¹⁻¹·min⁻¹ respectively, with succinate as substrate. Thus, both state 3 and state 4 respiratory activities were higher even than the highest control values. The possibility thus exists that there could be a certain degree of partial ischemia compatible with preserved mitochondrial function and rapid recovery of electrocortical activity, even when the remaining blood flow to the tissue is insufficient to supply oxygen and substrate for the maintenance of electrocortical activity during the ischemic period.

Respiratory Activities Related to Mitochondrial Protein

Figure 4 gives the state 3 as well as the state 4 respiratory activities related to the protein concentration. The pattern of changes was essentially the same as when cytochrome (a + a₃) was used as reference (cf. figs. 2 and 3). Thus, the slight discrepancy in total protein and total cytochrome (a + a₃) yields did not affect the basic results.

Discussion

As mentioned in the introduction, there is evidence that recovery of cerebral energy metabolism is significantly better following 30 min of complete compression ischemia than following 30 min of incomplete hypotensive ischemia. This difference may not be due to the absence or presence of a trickling blood supply to the brain, but rather to undefined factors related to the fact that one model (incomplete ischemia) leads to gross changes in the systemic circulation caused by the induced hypovolemic hypotension during the ischemic period. Furthermore, the models used differs in that complete compression ischemia leads to an essentially "bloodless" ischemia. The objective of the present experiments, which concern respiratory activity of mitochondria in vitro, was to shed further light on the mechanisms responsible for this difference. The same models for transient ischemia as in the previous metabolic studies, were used, except for induction of anesthesia. In the present work halothane was not
used. Even small doses of halothane were shown to influence mitochondrial respiratory activity, which could be an in vitro effect (Mela and Wrobel-Kuhl, unpublished data). In control experiments (not reported) we found no differences in mitochondrial function if the animals were decapitated without anesthesia, or if they were anesthetized with nitrous oxide as in the present work.

Studies of cerebral blood flow during incomplete ischemia of the present type have shown regional differences with the most pronounced decrease in CBF occurring in cortical tissue (reduced to about 5% of normal), while the CBF values for basal ganglia and brain stem were reduced to a lesser extent.17 In the present investigation brain mitochondria were isolated from the whole brain, except cerebellum and medulla oblongata. However, the demonstrated differences in recovery of mitochondrial function are in good agreement with results on recovery of the cerebral energy state (see above).

Since light and electron microscopic studies have shown early structural mitochondrial changes during anoxia and ischemia,18 the differences in total protein yields in the present material could indicate a loss of mitochondria during the isolation procedure, implying a greater vulnerability due to structural changes during ischemia in both models. If so, this seems to be reversible at least in complete ischemia. However, the calculated respiratory rates are expressed per mol cytochrome (a + a₃) or per mg of protein in the reaction mixture, and thus can be assumed to reflect the functional ability of the least damaged mitochondria. The differences in protein yields could also be explained by variable degrees of non-mitochondrial contamination in the preparations. This should not, however, influence the yield of cytochrome (a + a₃) and thus not the respiratory rates calculated as mol oxygen utilized per mol of cytochrome oxidase per min.

Pattern of Changes in Mitochondrial Function during Ischemia, and Following Recirculation

The respiratory control ratios (RCR), i.e. the ratio between substrate-plus ADP-stimulated and substrate (only) stimulated oxygen consumption rates, were measured. The symbols denote individual values; the mean values are represented by horizontal lines. **P < 0.01 indicates statistical significant differences from controls.

### Table 3

<table>
<thead>
<tr>
<th>Glutamate + malate</th>
<th>Control</th>
<th>Complete ischemia</th>
<th>Complete ischemia Recirculation</th>
<th>Incomplete ischemia</th>
<th>Incomplete ischemia Recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCR</td>
<td>6.5 (5.1–8.4)</td>
<td>3.3 (2.7–3.6)**</td>
<td>5.8 (4.3–6.6)</td>
<td>4.2 (3.0–5.3)*</td>
<td>3.5 (3.1–4.0)**</td>
</tr>
<tr>
<td>ADP/O</td>
<td>2.7 (2.5–3.0)</td>
<td>2.4 (1.7–2.6)</td>
<td>3.0 (2.7–3.5)</td>
<td>2.5 (2.0–2.8)</td>
<td>2.2 (2.0–2.3)**</td>
</tr>
<tr>
<td>Succinate</td>
<td>4.3 (3.2–5.3)</td>
<td>4.1 (3.0–5.1)</td>
<td>3.7 (3.4–4.1)</td>
<td>3.3 (2.6–4.2)</td>
<td>2.3 (1.9–2.8)**</td>
</tr>
<tr>
<td>ADP/O</td>
<td>1.8 (1.3–2.1)</td>
<td>1.4 (1.3–1.5)</td>
<td>1.8 (1.6–2.0)</td>
<td>1.3 (0.9–1.7)</td>
<td>1.2 (0.9–1.4)*</td>
</tr>
</tbody>
</table>

*P < 0.05, **P ≤ 0.01 indicate statistical significant differences from controls.
and Nicklas and well in the range of the results from Dr. Mela's laboratory. However, the control RCR-values differed from those reported for the rabbit and gerbil brains. These differences are probably due to species differences.

In the present study, the most pronounced changes occurred in state 3 (substrate + ADP stimulated) respiration. The changes in respiratory control ratio (RCR), were a result of the inhibition of state 3 respiration, and not of true uncoupling (RCR values approaching 1, were not found in any of the experiments). However, changes also occurred in the maximal phosphorylation rate (rate of ATP production), which is the product of the ADP/O ratio and the ADP-stimulated oxygen consumption rate.

Complete Ischemia. Following 30 min of complete ischemia there was a highly significant inhibition of state 3 respiration (and thereby also a reduction in RCR), which was reversed upon recirculation. ADP/O ratio showed a significant reduction during ischemia only when succinate was used as substrate. The inhibition of state 3 respiratory rates appeared greater with glutamate plus malate as substrates (50%) than with succinate (40%). The results obtained in complete ischemia demonstrate that recovery of mitochondrial function is possible after fairly short periods of recirculation, even when a pronounced inhibition of respiratory function occurs during the ischemia.

Incomplete Ischemia. During incomplete ischemia changes in state 3 respiratory activity, RCR and ADP/O ratio were not different from those observed in complete ischemia. If anything, changes occurring during incomplete ischemia appeared somewhat less

| Table 4 | The Influence of 30 min of Complete Ischemia, Induced by Decapitation, on Respiratory Control (RCR) and ADP/O Ratio, State 3 and state 4 Respiratory Activities (mol O2·mol (a + a3)−1·cm−1·min−1) in Brain Mitochondria from 3 Individual Rats. After Decapitation the Heads Were Kept at 37°C Before Isolation of the Mitochondria. Values in Brackets Represent State 3 Respiratory Activities After Addition of Mg++.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Glutamate + malate</th>
<th>ADP/O</th>
<th>State 3</th>
<th>State 4</th>
<th>RCR</th>
<th>ADP/O</th>
<th>State 3</th>
<th>State 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate + malate</td>
<td>2.8</td>
<td>2.3</td>
<td>184 (213)</td>
<td>65.4</td>
<td>2.9</td>
<td>1.2</td>
<td>159</td>
<td>55.7</td>
</tr>
<tr>
<td>Glutamate + malate</td>
<td>3.4</td>
<td>2.5</td>
<td>204 (227)</td>
<td>59.5</td>
<td>3.3</td>
<td>1.3</td>
<td>184</td>
<td>56.6</td>
</tr>
<tr>
<td>Glutamate + malate</td>
<td>3.4</td>
<td>2.6</td>
<td>218 (255)</td>
<td>64.2</td>
<td>2.6</td>
<td>1.5</td>
<td>173</td>
<td>66.3</td>
</tr>
<tr>
<td>Mean</td>
<td>3.2</td>
<td>2.5</td>
<td>202 (234)</td>
<td>63.0</td>
<td>2.9</td>
<td>1.3</td>
<td>172</td>
<td>59.5</td>
</tr>
</tbody>
</table>
pronounced. However, recirculation did not lead to normalization but rather to aggravation of the changes observed.

Maximal Phosphorylation Rates in Complete and Incomplete Ischemia. Figure 5 gives the calculated maximal phosphorylation rates (with glutamate plus malate as substrates) in the different experimental situations. Although a pronounced decrease was found during the ischemic period, recovery was found only during recirculation after complete ischemia. Following incomplete ischemia, the maximal phosphorylation rate was further reduced to about 30% of the normal value. In this situation, the capacity of mitochondria to generate ATP is thus severely reduced, even if optimal conditions are at hand.

Possible Mechanisms of Mitochondrial Damage

In the present study we used an in vitro technique to study changes in mitochondrial function inflicted during transient brain ischemia in vivo situations. Thus the finding of a significant impairment of mitochondrial respiratory function in vitro, i.e. after replenishment of oxygen and substrates, in the preparations from ischemic brains suggests that hydrolytic processes take place during ischemic conditions leading to degradation of mitochondrial membranes and/or structural alterations in membrane-bound enzymes. Evidently a recirculation period (in vivo) is necessary for reversal of the damage implying that synthetical mechanisms may be important. The reason for persistent inhibition of mitochondrial func-

![Graph](image-url)
tion during recirculation following incomplete ischemia is, however, unsettled.

Earlier reports have suggested that, during ischemia, there may be loss of mitochondrial-bound Mg++, necessary for oxidative phosphorylation.10,12 However, this does not seem to explain the inhibition of state 3 rates during the 30 min ischemic period, or the difference in functional recovery upon recirculation, as the pattern of changes was not influenced by Mg++ additions to mitochondrial preparations prior to the assay of state 3 activities.

Prounced tissue acidosis could be a factor of importance in promoting cellular damage,10,21 possibly in connection with lysosomal mechanisms.22 Furthermore, acidosis has been shown to inhibit mitochondrial respiratory function in vitro, especially if mitochondria are incubated in the presence of lysosomal enzymes.23 Since there is some remaining blood flow in incomplete ischemia, continued supply of glucose to the tissue causes a more pronounced lactate accumulation than in complete ischemia.5,7 However, acidosis cannot be the only factor of importance since inhibition of mitochondrial function was similar at the end of 30 min of complete and incomplete ischemia in spite of the fact that the acidosis is considerably more pronounced in the latter.

Pathophysiological Events in Ischemia — Implication of Present Findings

The phosphorylation mechanism of brain mitochondria has been shown to be very labile and appears to be more sensitive than in many other organs.24 The present results demonstrate that, following 30 min of incomplete ischemia, there is inhibition of mitochondrial function chiefly affecting state 3 respiration and maximal phosphorylation rate. However, this inhibition appears to be potentially reversible (cf. complete ischemia). It therefore seems likely that the pronounced reduction in state 3 respiration and maximal phosphorylation rate that is observed after 30 min of recirculation is a result of events occurring during this period. We envisage that one of 2 mechanisms is responsible. First, there may be an inadequate recirculation following restoration of normal perfusion pressure, adding a secondary ischemic insult to the initial one. If this is so, persisting energy failure and cellular acidosis could aggravate the mitochondrial damage, e.g. by lysosomal degradation of mitochondrial membrane. Second, if recirculation is adequate to cover the energetic needs of the tissue one must postulate that during recirculation, biochemical mechanisms come into play that prevent restoration of mitochondrial function and/or aggravate the damage inflicted during the initial ischemia. However, since these hypothetical mechanisms do not operate following complete ischemia, they must be facilitated by the conditions prevailing during incomplete ischemia. Apart from allowing supply of glucose, a circulation remaining during incomplete ischemia permits oxidative reactions to continue, albeit at a slow rate, and there is the possibility that the tissue looses factors that prevent oxidative damage from occurring upon reoxygenation.

If ischemic and/or post-ischemic damage involves degradation of membrane phospholipids by peroxidative mechanisms (see Demopoulos et al.), such factors could be antioxidants or free radical scavengers.

Presently, there is insufficient information to decide on the most likely mechanisms involved. However, in view of the possibility that recirculation may not be too dissimilar following complete and incomplete ischemia of the present types,10 it seems highly warranted to explore possible mechanisms of oxidative damage under the conditions of pronounced incomplete ischemia.

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References

THE ROLE OF continuous cerebrospinal fluid (CSF) drainage in the treatment of acute cerebral ischemia has not been defined. A recent report by Smialek et al. suggests that CSF drainage substantially reduces mortality in gerbils following bilateral carotid artery occlusion. The effect, if any, on acute focal ischemia has not been studied.

Hyperosmolar agents, including mannitol, glyceral, and low molecular weight dextran, have a beneficial effect when administered shortly following the onset of ischemia. However, previous studies suggest that protection is limited to the period of elevated plasma osmolality. Consequently, the use of hyperosmolar agents alone may not constitute a definitive form of therapy.

The object of this investigation was to study the effect of continuous CSF drainage, alone and in combination with mannitol, upon the development of cerebral infarction in conscious cats following MCA occlusion.

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Treatment of Acute Focal Ischemia with Continuous CSF Drainage and Mannitol

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SUMMARY A simple implanted device was used to occlude acutely the left middle cerebral artery (MCA) of 32 conscious cats. Groups of 8 cats each were treated with continuous cerebrospinal fluid (CSF) drainage, mannitol (1 gm/kg i.v.), or a combination of continuous CSF drainage and mannitol (1 gm/kg i.v.). Eight cats served as a control group. The neurological status of cats treated with mannitol improved transiently. Perfusion with a mixture of colloidal carbon and buffered paraformaldehyde was carried out 12 hours following MCA occlusion. Gross swelling of cerebral tissue, distribution of colloidal carbon, and breakdown of the blood-brain barrier to fluorescein were similar in the 4 groups. Reduction of mean capillary luminal diameter to 4.5 ± 1.0 μ (control 6.5 ± 1.0 μ) in the left Sylvian cortex was unaltered by treatment. A significant difference in the distribution of severe neuronal alterations was not demonstrated.

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Methods

Implantation of Occluding Device

Details of the implantation of the device used to occlude the MCA have been described previously. Thirty-two adult cats with a mean weight of 3,200 gms were anesthetized with sodium pentobarbital (30 mgm/kg) injected intraperitoneally. The left MCA of each cat was exposed through a transorbital approach and the slotted housing of the occluding device, with the short stylet inserted, was applied to its proximal segment. The orbit was briefly sprayed with neosporin aerosol. The small craniectomy was packed with small pieces of gelfoam and covered with a piece of thin silastic sheeting. The orbit then was filled completely with rapidly hardening epoxy cement. The small cranietomy was packed with small pieces of gelfoam and covered with a piece of thin silastic sheeting. The orbit then was filled completely with rapidly hardening epoxy cement. The incision was closed with 4-0 silk suture and a dressing applied. A 20-gauge polyethylene catheter was inserted into the left femoral vein through a small incision in the groin. The injection nozzle of the catheter was brought out through another small incision on the flank. The wounds were closed with 4-0 silk suture and dressings applied. Each animal was given 30 ml of isotonic saline by intravenous injection.

Occlusion of the MCA

The left MCA of the 32 conscious, unanesthetized
Recovery of brain mitochondrial function in the rat after complete and incomplete cerebral ischemia.

S Rehncrona, L Mela and B K Siesjö

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