Hyperbaric oxygenation affects rat brain function after carbon monoxide exposure

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Rogatsky GG, Meilin S, Zarchin N, S.R. Thom, Mayevsky A. Hyperbaric oxygenation affects rat brain function after carbon monoxide exposure. Undersea Hyperbaric Med 2002; 29(1):50-58. The application of hyperbaric oxygenation (HBO\textsubscript{2}) has been recommended for correction of neurological injury in severely CO-poisoned patients. However, the mechanisms of HBO\textsubscript{2} action on brain mitochondrial function under the circumstances is not yet understood completely. In the present study, the effect of HBO\textsubscript{2} on the rat brain after CO exposure was evaluated by measuring the intramitochondrial NADH and its responses to anoxic test or repetitive induction spreading depression (SD) leading to brain activation. A unique monitoring system for bilateral monitoring of brain NADH redox state was used. Rats were exposed to 3000 ppm CO for 30 (group A) or 60 min (C). In groups B and D, after CO exposure, the rats were exposed to HBO\textsubscript{2} (3 atm abs for 30 min). Following CO exposure in groups A and C, a definite decrease in the amplitude of the NADH response and significant increase in the number of waves of NADH was noted during induced cortical SD. Anoxic test in these two groups led to a significant decrease of maximum levels of NADH (reduction) at the end of observation. The amplitude, and the number of SD waves and magnitude of NADH deviation during anoxic test in group B after application of HBO\textsubscript{2}, was not significantly different from the values measured under the initial conditions. However, in group D, tendency of maintenance of the parameter’s initial level was weaker or absent. The results obtained indicated that suppression of brain energy metabolism is a characteristic manifestation of CO poisoning in rats. Restoration of cerebral energy metabolism by adequate dosage of HBO\textsubscript{2} may become an important factor for recovery of brain activities after CO poisoning.

carbon monoxide poisoning, spreading depression, NADH redox state, hyperbaric oxygenation, anoxia

INTRODUCTION

Change in the state of consciousness and/or neurological abnormalities is one of the important indications for application of HBO\textsubscript{2} in treatment of acute carbon monoxide poisoning (1-5).

Studies on animals have suggested that these neurological disturbances may be related to both a hypoxic insult, such as the result of COHb formation (6), as well as an ischemic insult due to carboxymyoglobin-induced cardiovascular dysfunction (7,8). At the same time, in the experiments it is known that intracellular uptake of CO can directly impair cerebral energy metabolism, inhibition of mitochondrial electron transport in the brain (9,10). Besides, toxic exposure to CO also produce significant oxidative stress in the brain, as indicated by increased lipid peroxidation (11,12), increased hydrogen peroxide production, intramitochondrial glutathion depletion, and nonenzymatic hydroxylation of salicylate (13).
Hypoxic and also this direct adverse effect of CO on cerebral energy metabolism was ameliorated by treatment of animals with HBO₂ (10,14,15). However, the mechanisms of HBO₂ action on brain mitochondrial function under the circumstances is not yet understood completely.

We have shown that the nature of the response to a brain activation event induced by cortical spreading depression (SD) could serve as an indicator to the integrity of the cerebral cortex under various conditions (16,17). During the SD event, a large increase in extracellular K⁺ occurs, leading to the activation of the Na⁺K⁺ATPase. In order to restore the ionic homeostasis, the energy metabolism is stimulated and the turnover of ATP is much higher as compared to the baseline activity.

In the present study, in order to test the effects of HBO₂ treatment on cerebral energy metabolism after CO exposure, we analyzed the capacity of the brain to respond to brain activation induced by SD.

**MATERIALS AND METHODS**

In the present study, we used an approach for bilaterally monitoring NADH redox state in awake rats under brain activation induced by cortical spreading depression (SD) (16,18) before and after CO exposure.

In previous studies we showed that under SD, the mitochondrial NADH was oxidized, meaning that production of ATP was stimulated. If the SD was induced in partial ischemic brain (17), the NADH response was completely different, namely, the amplitude of the NADH oxidation (decrease) was smaller or even the direction of the change was reversed. Under these conditions, the NADH will increase during the SD cycle. Therefore, the SD cycle can be used as a test for the integrity of the CBF autoregulation mechanism in the brain. The correlation between oxygen consumption and CBF under SD was described in detail (19). It was shown that the pumping of K⁺ into the cells was the stimulation of energy metabolism. The response of the brain to anoxia can also serve as an indicator of the integrity of the respiratory chain in the mitochondria. The amplitude of the NADH increase during anoxia will decrease when the mitochondria is damaged and the NADH was elevated before exposure to anoxia. Since the total increase in the NADH level is constant, the more it increases during CO exposure, the less it will increase during the anoxic test.

The principle of NADH monitoring from the surface of the brain is that excitation light (366 nm) is passed from the fluorometer to the brain via a bundle of optical fibers made of quartz. The emitted light (450 nm), together with the reflected light at the excitation wavelength, is transferred to the fluorometer via another bundle. The changes in the reflected light are correlated to changes in tissue blood volume and also serve for correction of hemodynamic artifacts appearing in the NADH measurement. The changes in fluorescence and reflectance signals are calculated relative to the calibrated signals under normoxic conditions (16,18). This type of calibration is not absolute, but provides reliable and reproducible results from different animals and between different laboratories. These measurements were made inside a hyperbaric chamber so that HBO₂ treatment after CO exposure was possible (20). In the monitoring approach used, a light guide was implanted above the two hemispheres of the brain together with 2 pairs of ECoG (electrocorticogram) electrodes (Fig. 1).
A push-pull cannula for KCl application (induction of SD) was implanted 2 mm from the light guide holders (16,21,22). Rats were exposed to 3000 ppm CO for 30 min (group A, n=8) or for 60 min (group C, n=8). Two other groups of rats were exposed to 3000 ppm CO for 30 or 60 min followed by 30 min HBO₂ at 3 atmospheres absolute (atm abs) in a Bethlehem Steel Corp. FM-21-A chamber (group B, n=6 and group D, n=8, respectively). SD was induced before and 20 min, 2, 3, and 4 hours after CO exposure as well as after HBO₂ treatment. The amplitude of the NADH response to SD was found to be affected by the treatments. At the beginning and end of each experiment, an anoxic test (spontaneously breathing 100% nitrogen) was done in order to measure the maximal deviation of CF (increase of NADH). The purpose of this test was to evaluate the mitochondrial energy state in the brain (18,20,23). Fig. 2 shows typical NADH fluorometry and ECoG responses to anoxic test and KCl application leading to SD cycles.

**FIG. 2** - Metabolic and electrical responses to anoxic test (A), cortical spreading depression (SD) during control (B) and after 3000 ppm CO exposure (C). R, F, CF, reflectance, fluorescence and corrected NADH fluorescence recorded from right (R) and left (L) hemisphere. ECoG, electrocorticogram
Fig. 3 describes the various protocols of the experimental groups.

**FIG. 3** — Details of the various protocols in the experimental groups A, B, C and D.

**RESULTS**

Fig. 4 represents an example of typical changes of NADH waves, induced by KCl application after CO exposure.

**FIG 4** — Typical gradual increase of the number of waves SD (CF) in experimental groups A and C. A, B, C, D, E – the induction of SD before or 20 min, 2, 3 and 4 h after CO exposure, accordingly
These changes can be described as a trend toward decrease in the amplitude of the NADH response and an increased number of cortical SD waves upon a single passage of KCl solution. In this study, we have found that following CO exposure of groups A and C (control groups), an obvious and significant decrease in the amplitude of NADH waves of cortical SD was recorded (Fig. 5, groups A, C).

As one can see from the histogram, the decrease of the amplitude in group C is more pronounced than in group A. Use of HBO₂ after CO exposure accompanied a maintenance of the initial level of NADH amplitude in group B. This effect of preservation of the wave amplitude of SD was absent in the rats of group D.

![Histogram showing NADH amplitude changes](http://rubicon-foundation.org)

**FIG. 5** — Histogram presenting the amplitude of NADH waves of cortical spreading depression (SD), induced by passage of 1% KCl solution before and after CO exposure in control groups A and C (n = 8 and 8, respectively), as well as after HBO2 treatment groups B and D (n = 6 and 8, respectively). Abscissa is the time (hours) after CO exposure and ordinate is amplitude of CF (NADH in % of control). *P<0.05 compared with control.

The number of SD waves after CO exposure is presented in Fig. 6. There is a clear tendency toward an increase in this parameter in the control groups (Fig. 6, groups A, C). However, after HBO₂ application, the significant increase of SD wave number was not observed in group B (Fig. 6, group B). In group D, this effect of HBO₂ protection was absent (Fig. 6, group D).

The anoxic test in the control groups A and C shows a significant decrease of maximal deviation levels of NADH (reduction) at the end of the observation in comparison to the initial experimental period (Table 1, groups A, C). This parameter was not different in group B from
the magnitude of NADH deviation at the beginning of the experiments (Table 1, group B). The magnitude of NADH deviation by anoxic test at the end of the experiments in group D remained significantly lower as compared to the initial state (Table 1, group D). This parameter in the untreated group was invariable (Table 1, “Untreated”).

![Graph](image)

**FIG. 6** — Histogram presenting the number of NADH waves due to cortical SD, induced after single passage of KCl solution. The ordinate presents the number of NADH waves in % of control. Other designations are as in Fig. 3.

**Table 1: Effects of 3000 ppm CO on the magnitude of NADH deviation during anoxic test in the various experimental groups (% of initial state).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial State</th>
<th>n</th>
<th>Terminal State</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100 ± 4.7</td>
<td>5</td>
<td>99.1 ± 3.35</td>
<td>5</td>
</tr>
<tr>
<td>A (30’ CO)</td>
<td>100 ± 8.3</td>
<td>14</td>
<td>60.8 ± 3.3*</td>
<td>13</td>
</tr>
<tr>
<td>B (30’ CO+HBO₂)</td>
<td>100 ± 6.2</td>
<td>13</td>
<td>92.2 ± 3.2</td>
<td>10</td>
</tr>
<tr>
<td>C (60’ CO)</td>
<td>100 ± 6.8</td>
<td>13</td>
<td>49.59 ± 1.24*</td>
<td>12</td>
</tr>
<tr>
<td>D (60’ CO+HBO₂)</td>
<td>100 ± 8.6</td>
<td>11</td>
<td>67 ± 4.6*</td>
<td>9</td>
</tr>
</tbody>
</table>

Untreated, A, B, C, D – groups of experiments; Initial state – before CO exposure; Terminal state animal’s death. Mean ± SE; *P < 0.05 compared with initial state.
DISCUSSION

In the present study, NADH redox state was used in order to evaluate the responses to CO exposure. The interpretation of the results is somewhat limited since cerebral blood flow (CBF) and cerebral blood volume (CBV) were not monitored.

Progressive decrease in the amplitude and an increased number of NADH waves upon a single passage of KCl solution in control groups (A and C) combined with the decrease in the maximal deviation (reduction) of NADH level upon the anoxic test, have been interpreted as manifestation of mitochondrial dysfunction. Probably one explanation for the character’s changing of the NADH waves may be in the compensatory increase of number SD required for elimination of local ionic disbalance and reactivation of transmembrane ionic transport, particularly after CO induced decrease in energetic status of mitochondria.

The results obtained in group B show that the development of disbalance in energy metabolism in the cerebral cortex can be delayed or prevented by HBO\textsubscript{2} treatment. However, whereas a complete restoration of studied parameters was observed in group B, group D did not exhibit tendency to maintain these parameter levels. These data indicate that the restoration of NADH parameters upon HBO\textsubscript{2} application is dependent on the overall severity of hypoxic and cellular disturbances in the brain, which in group D was more pronounced due to the twice greater duration of CO inhalation, relative to group B.

We propose that the signs of NADH dysfunction found by us can be caused by direct injurious action on the brain CO (9,10) and the lipid peroxidation (11,12), that probably inhibit mitochondrial electron transport. Displacement of CO via HBO\textsubscript{2} exposure from the proteins it has bound to (24-28) also provides the preconditions for the restoration of normal levels of electron transport along mitochondrial respiratory chain, because HBO\textsubscript{2} has some intracellular protective effect on the brain independently from increased O\textsubscript{2} carrying capacity (10,14,15,29).

The restoration of mitochondrial function upon HBO\textsubscript{2} exposure in our experiments was adequately reflected by the dynamics of those NADH parameters examined.

Notably, the anoxic test has proven in our studies to be a sensitive and quantitative indicator of mitochondrial respiration. As an alternative to the oxygen test (30), it provides an adequate prognostic value in the evaluation of damage, as well as a potential for restoration of mitochondrial energy metabolism.

Our data also suggest a role for HBO\textsubscript{2} as a means by which cerebral function may be restored after CO intoxication. Earlier it has been obtained that HBO\textsubscript{2} at 2.8-3 atm abs markedly accelerates protection against injurious sequelae occurring in CO poisoning in rats (14,15,24,31). However, comparison between the data in groups B and D suggest that the duration of HBO\textsubscript{2} exposure is probably also an important factor for optimizing the positive effect.

In recent studies it was revealed that HBO\textsubscript{2} at 3 atm abs for 60 min significantly increased the survival time and rate in rats poisoned with CO (2700 ppm, for 60 min) (31). Moreover, it was also established that exposure to hyperbaric O\textsubscript{2} at 3 atm abs for 1-2 h actually improves outcome in rats after acute cerebral ischemia (32-34). It is important to note that in these experiments, HBO\textsubscript{2} treatment regulated the brain energy metabolites (34) and prevented the aggravation of lipid peroxidation in the ischemic periphery (33).

Further investigation is necessary to establish the optimal therapeutic dose of HBO\textsubscript{2} treatment for recovery of the brain mitochondrial respiration after CO poisoning. In addition, it
will be important to monitor CBF and CBV, e.g. using laser Doppler flowmetry, in order to interpret the results in depth.

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REFERENCES