Combined administration of hyperbaric oxygen and hydroxocobalamin improves cerebral metabolism after acute cyanide poisoning in rats

M. B. Hansen,1,2 N. V. Olsen,3,4 and O. Hyldegaard1,2

1Laboratory for Hyperbaric Medicine, Department of Anesthesia, Centre of Head and Orthopedics, Copenhagen University Hospital, Rigshospitalet, Denmark; 2Hyperbaric Unit, Department of Anesthesia, Centre of Head and Orthopedics, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; 3Department of Neuroanesthesia, The Neuroscience Centre, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; and 4Department of Neuroscience and Pharmacology, University of Copenhagen, Copenhagen, Denmark

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Cyanide (CN) is one of the most potent and deadly poisons. Throughout history, CN has been used as an agent for suicide and homicide (37). Furthermore, the U.S. Government considers CN to be among the most likely agents of chemical terrorism (15). Fire accidents are the leading cause of CN poisoning, and ∼35% of fire victims have toxic blood CN levels (14, 32). CN is an important contributor to death by smoke inhalation, and the percentage of fatalities with lethal blood concentrations of CN ranges from 33 to 87% (2, 6).

Since CN is a small, lipid-soluble molecule, the distribution and penetration into cells is fast, with rapidly arising symptoms, including headache, nausea, neurological deficits, cardiovascular collapse, and death (42). Some of the symptoms observed in humans correspond well to symptoms seen in CN-poisoned rats (30). CN binds with high affinity to the enzyme cytochrome oxidase a1, the terminal enzyme complex of the respiratory chain in complex IV, thus blocking mitochondrial electron transport and oxidative phosphorylation (7), resulting in the depletion of adenosine triphosphate (ATP) and, therefore, cellular energy failure. As a consequence, energy supply shifts from aerobic to anaerobic metabolism. This results in metabolic acidosis as blood lactate concentrations increase to such an extent that the body’s buffering abilities are incapable of maintaining homeostasis (6).

The optimal treatment of acute CN poisoning remains unclear, and treatment protocols are based on decontamination, life support initiation, and early administration of an antidote (20). Hydroxocobalamin (OHCob) is widely used and seems to be the antidote of choice when acute CN poisoning is suspected (4, 19). Adjunctive hyperbaric oxygen therapy (HBOT) is recommended, especially in cases where supportive measures or antidotes fail (21, 29, 33). We have shown that acute CN poisoning leads to an immediate increase in cerebral interstitial concentrations of lactate and glucose (31). HBOT was as effective as OHCob in the amelioration of these glucose and lactate surges (31). Furthermore, in a previous model, we demonstrated that HBOT is able to increase whole blood CN concentration, suggesting that CN was displaced from the extravascular compartment to blood (30). OHCob, on the other hand, may act as a CN scavenger, thereby maintaining a prolonged effect on lactate and glucose metabolism during the observation period. This is not seen with HBOT, and lactate as well as glucose concentrations may rise once HBOT has been terminated (31). In contrast to OHCob, HBOT significantly increased the brain tissue partial oxygen pressure (Pto2) and decreased respiratory distress and cyanosis (31).

We aimed to evaluate the combined effect of OHCob and HBOT on interstitial surges of lactate concentrations in the rat brain during CN poisoning measured by means of microdialysis, the primary endpoint being the mean interstitial brain lactate concentration in the last part of the experiment [mean of sample was 10–21, i.e., time (t) of 135–300 min]. We hypothesized that the combined effect of HBOT and OHCob will prevent high interstitial brain lactate concentrations during acute CN intoxication (samples 3–9; t = 30–120 min) and keep steady, low lactate concentration during the observation period (samples 10–21; t = 135–300 min). In addition, we assessed the effects of OHCob and HBOT on interstitial brain...
glucose and glycerol concentrations, lactate-to-pyruvate (L/P) ratio, and the hemodynamic and respiratory response.

**METHODS**

**Study Subjects and Experimental Design**

Nonpregnant female Sprague-Dawley rats (Taconic) with normal physiological hormone cycle and weighing 240–280 g were fed a standard diet and water ad libitum. Euthanasia was performed by cervical dislocation in the anesthetized rat at the end of each experiment. The animal experimental study was based on a well established model (31), and all experiments were performed within the same circadian stage. All experiments were carried out in accordance with the National Institutes of Health Guide for care and use of laboratory animals and approved by a government-granted license from the Danish Animals Ethical Committee.

In a nonrandomized design, the rats were allocated into three experimental groups. Group A (n = 17) was composed of vehicle [1.2 ml isotonic NaCl intra-arterially (i.a.)]. Group B (n = 17) was composed of CN poisoning [5.4 mg/kg potassium CN (KCN) i.a., purchased custom-made from the Pharmacy of Rigshospitalet, Copenhagen, Denmark]. Group C (n = 17) was composed of CN poisoning (5.4 mg/kg KCN i.a.) and OHCob (100 mg/kg i.a.) and HBOT (284 kPa in 90 min).

Each experiment lasted 300 min. First, a microdialysis sample was collected at t = 0 min. At t = 15 min, the rats were given an infusion with either vehicle (group A) or KCN (groups B and C). The arterial catheter was flushed with 0.3 ml of saline to ensure no remaining KCN. The CN dose gives rise to an initial plasma concentration of ~120 μM (30), similar to toxic human exposure levels in fire accidents (6). The intervention (HBO and OHCob) was given at t = 30 min. HBOT finished at t = 120 min. The rats were then observed with continuous microdialysis sampling during the remaining part of the experiment (180 min). Categorical data regarding general symptoms were registered on a form during the CN intoxication and the subsequent observation period. The following variables were collected: seizures located to the leg where infusion was given or general seizures, respiratory distress, respiratory arrest, drop in mean arterial blood pressure (MAP) below 60 mmHg, and cyanosis.

**Preliminary Sham Intervention Experiment**

To verify that the changes measured in the microdialysate corre- lated to the effects induced by OHCob and HBOT during KCN intoxication and not to any other coincidental factor, a preliminary sham intervention experiment was conducted: sham (n = 6); OHCob (100 mg/kg i.a.) and HBOT (284 kPa in 90 min). It was found that neither the mean brain interstitial lactate and glycerol concentrations nor mean L/P ratios were affected by the administration of HBOT and OHCob. Glucose showed a significant increase during the intervention (samples 3–9: 0.39 ± 0.23 mM) compared with the control group A (samples 3–9: 0.29 ± 0.12 mM) receiving isotonic NaCl and no treatment (P < 0.007, unpaired t-test). This may be explained by the fact that cerebral glucose is closely related to the regional cerebral blood flow (16) and that infusion of OHCob may increase the blood flow in the brain (8). HBOT may partially counteract the effect of OHCob by reducing both the plasma glucose concentration (18) and to some extent cerebral blood flow (9, 10). A possible reduction in cerebral blood flow seemed to have no unwanted effects on the oxygen supply to brain tissue, since PtO₂ reached the same level as in group C. After HBOT was completed, both groups returned to the same baseline levels as in control group A.

**Rat Preparation**

The rats were anesthetized with a mixture of 0.315 mg/ml fentanyl, 10 mg/ml fluanizone, and 5 mg/ml midazolam (5 mg/ml hypnorm/ormhydrin). For induction, a dose of 0.3 ml/100 g was given subcutaneously. Anesthesia was maintained with 0.1 ml/100 g subcutaneously every 30 min. A temperature probe was placed in the vagina, and a temperature controller (CMA/150) maintained body temperature at an average of 37°C by means of a heating pad. Tracheotomy with polyethylene tubing was performed on all rats to ensure a secure airway. A polyethylene catheter (TYGON) was placed in the right femoral artery, connected to a pressure transducer (Edwards Life Sciences), and calibrated at the level of the heart. The catheter was then connected to a pump with continuous isotonic NaCl infusion of 1 ml/h. A polyethylene tube was placed subcutaneously on the back of the rat (regio lumbalis dexter), through which anesthésia supplements were given.

**Experimental Procedures**

**Stereotoxic surgery.** The rats were fixed in a stereotoxic instrument (CMA Model 51500) in prone position. Two small boreholes were drilled in the skull with a round bur (RA 1/021, Meisinger) according to the stereotoxic coordinates: AP = +3.0 mm anterior to bregma, L = ± 3 mm, and V = −1.0 mm (31, 34). Bregma was defined as the point of intersection of the sagittal suture with the coronal suture. Where the two sides of the coronal suture met the sagittal suture at different points, bregma was designated as midway between the two junctions. The coordinates were consistent to the frontal cerebral parenchyma near forceps minor of the corpus callosum and were chosen because of the increased activity of oxidative enzymes in that area during KCN intoxication (25). The borehole to the right of sutura sagittalis was used for the placement of the microdialysis catheter. A guide cannula was inserted into the hole and fixed with two anchor screws and dental zinc polycarboxylate cement (Poly-F Plus, Dutsply DeTrey). The borehole to the left of sutura sagittalis was used to place an oxygen probe (Licox Oxygen Probe REF CCF1, Integra) measuring brain PtO₂, connected to an oxygen monitoring system (Licox CMP Monitor, Integra). Thermocalibration of PtO₂ was ensured with the use of a temperature probe (Licox Temperature Probe REF C8, Integra) placed in the rectum. The PtO₂ catheter was allowed to stabilize for at least 1 h after insertion. PtO₂ was recorded every 15 min.

**Cerebral microdialysis.** Lactate, glucose, glycerol, and pyruvate were sampled in the dialysate using the CMA/102 Microdialysis Analyzer (CMA/Microdialysis, Stockholm, Sweden). The L/P ratio was calculated for each sample. When the pyruvate concentration was undetectable, half of the lower limit of detection was used (0.5 μM), in accordance to previous studies (22). The microdialysis probe (CMA 12; 4-mm tip length, PAES 100,000 Da) was inserted into the frontal cerebral parenchyma. The probe was perfused with CMA perfusion fluid (in mM: 147 NaCl, 2.7 KCl, 1.2 CaCl₂, 0.85 MgCl₂) at 2 μl/min using a CMA102 microinjection pump. After a 1-h equilibration period, samples of dialysate were collected every 15 min with a CMA142 microfraction collector, thus providing a sample volume of 30 μl/min. The samples were analyzed immediately after the experiment ended. A pilot study showed that the samples were insensitive to evaporation during the 90 min of HBOT.

**HBOT.** After preparation, the rat was transferred to a specially designed pressure chamber with a horizontal viewing port and placed on a circular platform with an integrated heating pad. At t = 30 min, the tracheal cannula was connected to the breathing system of the pressure chamber through a T-shaped tube, and oxygen was supplied immediately with a free-flow of 1,500–2,000 ml/min. The pressure chamber and breathing system have been described in details in previous studies (23, 24). The HBOT consisted of pressurization over 5 min to a pressure of 284 kPa (i.e., 2.8 atm absolute) during continued oxygen breathing (inspired O₂ fraction = 1.0). The pressure was applied for 90 min followed by 5 min of decompression.

During five pilot studies, the position of the microdialysis probe in the frontal cerebral cortex was confirmed by dissecting the brain, followed by visual inspection after each experiment. In addition, three
experiments verified the position of the probe by supplemental infusion of methylene blue through the microdialysis catheter followed by decapitation of the anesthetized rat, removal of the brain, and subsequent snap-freezing on dry ice. The placement of the catheter was verified by cutting slices of the frozen brain at the site of the catheter.

**Outcome and Sample Size**

Our primary endpoint was mean interstitial brain lactate concentrations in the last part of the experiment (mean of sample size of 10–21, i.e., \( t = 135–300 \) min). Secondary endpoints were mean interstitial brain glucose and glyceral concentrations, L/P ratios, \( P_{\text{O}_2} \) measurements, MAP, respiration, and cyanosis.

Based on previous reports using a similar model (31), we estimated a mean interstitial brain lactate concentration in our control group of \( 0.22 \pm 0.10 \) mM in the last part of the experiment (samples 10–21). A reduction of 0.12 mM in interstitial brain lactate concentration was considered clinically relevant. With \( \alpha = 0.05 \) and power of 90%, 15 rats would be required in each group.

**Data Analysis and Statistics**

Mean concentrations of lactate, glucose, glyceral, and L/P ratios were measured every 15 min during the 300-min observation period. The 21 samples were subdivided into two groups: samples 3–9 represented the administration of OHCob and HBOT, whereas samples 10–21 expressed the observation time from ended intervention until termination of the experiment. Sample 1 was considered to be a baseline value, whereas sample 2 expressed the effects of the KCN infusion without any treatment.

Statistical analyses were performed using Statistical Package for the Social Sciences 15.0 software (SPSS, Chicago, IL). Test for normality was done using Kolmogorov-Smirnov analysis. One-way ANOVA was used to compare the baseline values in the three groups and to assess whether the overall variation in the microdialysis outcomes were attributable to differences between the group means. Post hoc Bonferroni correction test was performed to allocate significant differences between subject groups. Continuous data are presented as means ± SD, unless otherwise indicated. Categorical data are reported as absolute numbers, and comparisons were done using Fisher’s exact test. \( P \) values of <0.05 were considered statistically significant.

**RESULTS**

In all, 69 rats were studied. Three rats died due to KCN poisoning in the KCN group (group B) at \( t = 30 \) min (\( n = 1 \)) and at \( t = 35 \) min (\( n = 2 \)). One rat died of KCN poisoning in the treatment group (group C) at \( t = 30 \) min. Data from these rats were excluded. Six rats were used in the preliminary sham experiment (see METHODS), and eight rats were used to verify probe position and quantification of dialysate evaporation. The remaining 51 rats were included in the three main groups (group A: \( n = 17 \); group B: \( n = 17 \); group C: \( n = 17 \)). At baseline, the groups did not differ from each other (Table 1).

**General Symptoms**

**KCN intoxication (\( t = 15 \) min).** In the acute phase of the KCN intoxication, the rats in group C (KCN + OHCob + HBOT) and group B (KCN) had general seizures (2 vs. 3 respectively; \( P = 1.00 \), Fisher’s exact test) or seizures located to ipsilateral leg of KCN infusion (14 vs. 12; \( P = 0.69 \), Fisher’s exact test), respiratory arrest (14 vs. 15; \( P = 1.00 \), Fisher’s exact test), and cyanosis (17 vs. 16; \( P = 1.00 \), Fisher’s exact test).

**Treatment (\( t = 30 \) min).** Once administration of OHCob and HBOT commenced, the rats in group C showed a significant improvement in respiration compared with the intoxicated rats in group B receiving no treatment (2 vs. 17, respectively; \( P < 0.0001 \), Fisher’s exact test). Likewise, a significant disappearance of cyanosis was observed in group C compared with group B (15 vs. 2; \( P < 0.0001 \), Fisher’s exact test).

**MAP and \( P_{\text{O}_2} \).**

**KCN intoxication (\( t = 15 \) min).** In the acute phase, the intoxication of groups B and C caused a drop in MAP from 91 ± 10 and 90 ± 9 mmHg to 57 ± 25 and 46 ± 5 mmHg, respectively (Fig. 1). There was no significant difference in the proportion of rats having a MAP of <60 mmHg between groups C and B (17 vs. 13; \( P = 0.1 \), Fisher’s exact test). No significant change in \( P_{\text{O}_2} \) was observed during KCN intoxication in both groups (Fig. 2).

**Treatment (\( t = 30 \) min).** Once treatment was initiated in group C, MAP improved, ranging from 134 ± 17 to 105 ± 17 mmHg, and a greater number of rats compared with group B remained hemodynamically stable (MAP above 60 mmHg) for the rest of the observation period without any fluctuations in MAP (13 vs. 3; \( P = 0.002 \), Fisher’s exact test). \( P_{\text{O}_2} \) increased from 35 ± 4 to 156 ± 37 mmHg. After HBOT ended, \( P_{\text{O}_2} \) returned to baseline levels in group C (Fig. 2).

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**Table 1. Baseline data at time = 0 min**

<table>
<thead>
<tr>
<th></th>
<th>Control (( n = 17 ))</th>
<th>KCN (( n = 17 ))</th>
<th>KCN + HBOT + OHCob (( n = 17 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, g</td>
<td>258 ± 17</td>
<td>258 ± 17</td>
<td>255 ± 9</td>
</tr>
<tr>
<td>Induction of anesthesia, ml</td>
<td>0.77 ± 0.05</td>
<td>0.77 ± 0.03</td>
<td>0.76 ± 0.03</td>
</tr>
<tr>
<td>Rectal temperature, °C</td>
<td>36.8 ± 0.7</td>
<td>37.1 ± 0.8</td>
<td>36.9 ± 0.6</td>
</tr>
<tr>
<td>Vaginal temperature, °C</td>
<td>36.8 ± 0.5</td>
<td>37.2 ± 0.3</td>
<td>36.9 ± 0.4</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>90 ± 13</td>
<td>91 ± 10</td>
<td>90 ± 9</td>
</tr>
<tr>
<td>( P_{\text{O}_2} ), Torr</td>
<td>38.6 ± 5.2</td>
<td>36.1 ± 2.8</td>
<td>35.4 ± 3.6</td>
</tr>
</tbody>
</table>

Data are means ± SD; \( n \), number of rats. KCN, potassium cyanide; HBOT, hyperbaric oxygen therapy; OHCob, hydroxocobalamin; MAP, mean arterial pressure; \( P_{\text{O}_2} \), brain tissue oxygen partial pressure. One-way ANOVA and post hoc Bonferroni test showed no significant difference in baseline data between the groups.

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Fig. 1. Mean arterial pressure (MAP) in rats allocated to (1) vehicle treatment \( [1.2 \text{ ml of isotonic NaCl at time } (t) = 15 \text{ min}] \); (2) KCN poisoning \( [5.4 \text{ mg/kg at } t = 15 \text{ min}] \); (3) KCN poisoning receiving hydroxocobalamin \( [100 \text{ mg/kg at } t = 30 \text{ min}] \) and hyperbaric oxygen therapy (HBOT; 284 kPa in 90 min, \( t = 30–120 \) min). Values are means ± SE.
Intracerebral Microdialysis

Mean interstitial concentrations of lactate, glucose, glycerol, and L/P ratios for groups A, B, and C are summarized in Table 2.

**Lactate.** Lactate was statistically significantly different among the three groups for samples 3–9 [ANOVA, F(2, 330) = 158.65; P < 0.0001] and samples 10–21 [ANOVA, F(2, 516) = 94.75; P < 0.0001]. The control group A demonstrated a steady interstitial lactate concentration ranging from 0.17 ± 0.07 to 0.25 ± 0.13 mM, whereas rats given KCN infusion (t = 15 min) experienced a marked increase in lactate concentration reaching a maximum mean value of 0.93 ± 0.23 mM (t = 30 min) (Fig. 3). From here, the lactate concentration started to decrease and reach baseline values (t = 105 min). In KCN-poisoned rats given OHCob (t = 15 min) and HBOT (t = 30–120 min) subsequently, the surge in lactate was attenuated during the treatment period (samples 3–9) compared with rats given KCN infusion alone (0.10 ± 0.06 vs. 0.55 ± 0.33 mM; P < 0.0001, post hoc Bonferroni test). In addition, the effects of the treatment interventions were sustained for the rest of the experiment, i.e., samples 10–21 (0.08 ± 0.06 vs. 0.23 ± 0.16 mM; P < 0.0001, post hoc Bonferroni test).

**Glucose.** Glucose was statistically significantly different among the three groups for samples 3–9 [ANOVA F(2, 343) = 26.6; P < 0.0001] and samples 10–21 [ANOVA F(2,486) = 45.98; P < 0.0001]. The control group A had almost constant mean interstitial glucose concentration with a slight decrease in the last part of the experiment (Fig. 4). Unlike the control group A, the KCN-poisoned rats presented with an increase in glucose concentrations obtained from 30 to 120 min (samples 3–9). The surge in glucose was, however, more pronounced in rats without OHCob infusion and HBOT. During the observation period (samples 10–21), the glucose concentration remained low in KCN-poisoned rats treated with OHCob and HBOT compared with the control group (mean 0.12 ± 0.08 vs. 0.23 ± 0.14 mM; P < 0.0001, post hoc Bonferroni test) and KCN-poisoned rats receiving no treatment (0.12 ± 0.08 vs. 0.26 ± 0.18 mM; P < 0.0001, post hoc Bonferroni test).

**Glycerol.** Glycerol was statistically significantly different among the three groups for samples 3–9 [ANOVA F(2, 283) = 57.86; P < 0.0001] and samples 10–21 [ANOVA F(2,377) = 26.12; P < 0.0001]. The control group did not experience any noticeable changes in interstitial glycerol concentrations during the experiment (Fig. 5). The KCN-poisoned rats without treatment showed an increase in glycerol concentrations, whereas the rats given the treatment conversely showed a decrease in glycerol concentrations during the acute phase of the CN intoxication.

Table 2. Mean interstitial brain lactate, glucose, and glycerol concentrations and L/P ratios

<table>
<thead>
<tr>
<th></th>
<th>Lactate, mM</th>
<th>Glucose, mM</th>
<th>Glycerol, mM</th>
<th>L/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples 3–9</td>
<td>Samples 10–21</td>
<td>Samples 3–9</td>
<td>Samples 10–21</td>
</tr>
<tr>
<td>Control (n = 17)</td>
<td>0.19 ± 0.010</td>
<td>0.22 ± 0.11</td>
<td>0.29 ± 0.12</td>
<td>0.26 ± 0.18</td>
</tr>
<tr>
<td>KCN (n = 17)</td>
<td>0.55 ± 0.33</td>
<td>0.23 ± 0.16</td>
<td>0.51 ± 0.28</td>
<td>0.23 ± 0.14</td>
</tr>
<tr>
<td>KCN + HBOT + OHCob (n = 17)</td>
<td>0.10 ± 0.06</td>
<td>0.08 ± 0.06</td>
<td>0.40 ± 0.24</td>
<td>0.12 ± 0.08</td>
</tr>
</tbody>
</table>

Data are means ± SD; n, number of rats. L/P ratio, lactate/pyruvate ratio; samples 3–9, samples taken from time (t) = 30–120 min (during OHCob infusion and HBOT); samples 10–21, samples taken from t = 135–300 min (observation period). Significance difference between control and KCN (P < 0.0001). Significance difference between KCN + HBOT + OHCob and control and KCN (P = 0.002 and P < 0.0001, respectively). Significance difference between KCN + HBOT + OHCob and control and KCN (P = 0.002 and P < 0.0001, respectively). Significance difference between KCN + HBOT + OHCob and control and KCN (P = 0.002 and P < 0.0001, respectively). Significance difference between KCN + HBOT + OHCob and control and KCN (P = 0.007). Significance difference between KCN + HBOT + OHCob and control and KCN (P = 0.001 and P < 0.0001, respectively). Significance difference between control and KCN (P = 0.002). Significance difference between control and KCN (P < 0.0001). Significance difference between control and KCN (P < 0.0001).
intoxication compared with the group giving no treatment, i.e., samples 3–9 (8 ± 4 vs. 21 ± 11; \( P < 0.0001 \), post hoc Bonferroni test). The concentrations were persistently low during the last part of the experiment in rats receiving treatment compared with the control group (6 ± 3 vs. 14 ± 12; \( P < 0.0001 \)) and the KCN-poisoned rats receiving no treatment (6 ± 3 vs. 10 ± 6; \( P < 0.0001 \), post hoc Bonferroni test).

**L/P ratio.** L/P ratio was statistically significantly different among the three groups [ANOVA \( F(2,341) = 6.24; P < 0.0001 \) and samples 10–21 [ANOVA \( F(2,525) = 15.66; P < 0.0001 \)]. Both groups B and C of KCN-poisoned rats showed an increase in mean interstitial L/P ratio after the acute intoxication (Fig. 6). The rats treated with OHCob and HBOT experienced a subsequent reduction in L/P ratio after HBOT initiation (\( t = 30 \) min). The effect persisted throughout the rest of the experiment and was significantly smaller than in intoxicated rats receiving no treatment (0.14 ± 11 vs. 0.25 ± 0.27 mM; \( P < 0.0001 \), post hoc Bonferroni test).

**DISCUSSION**

Although the brain only represents a small percentage of the total body weight, it utilizes 25% of the glucose (38) and is accountable for 20% of the total oxygen consumption and dependent on oxidative metabolism. In this sense, the brain is an extremely exposed organ, especially during KCN poisoning.

**Microdialysis**

In this report, we studied the combined effects of OHCob and HBOT on the KCN intoxicated rat brain using an already well established model on cerebral microdialysis (31). As previously stated, we used a flow rate of 2 \( \mu \)l/min of the microdialysate central nervous system (CNS) perfusion fluid to ensure a sufficient amount of dialysate during the 90-min sampling period of HBOT in the pressure chamber where the microdialysis sampler was inaccessible. Since a high perfusion rate of dialysate may be liable to decrease the recovery rates of the cerebral extracellular substances (38), a CMA 12 (4-mm tip length) probe with a long membrane and a high molecular cutoff to optimize recovery was chosen. Furthermore, the insertion of a probe into the brain tissue will cause micro lesions, which may influence the concentrations in that area for a certain period of time. According to manufacturer guidelines, we addressed these concerns by having an hour of consistent microdialysate fluid perfusion to obtain baseline values.

Since CN binds universally to tissue cells after i.a. infusion and lactate crosses the blood-brain barrier (3, 17), control experiments were performed in our previous report (31), where the effects of i.a. KCN infusion on interstitial cerebral lactate concentrations were tested against i.a. lactate infusions. A quantitative as well as a qualitative deviation in the behavior of cerebral lactate concentrations during KCN infusions, compared with lactate infusions, was seen, indicating that the surges in metabolites seen in the cerebral microdialysate are due to changes in the metabolic state of the brain cells rather than influenced by influx of lactate from plasma and other tissue compartments (31).

**KCN Intoxication**

In the present experiments, intoxication was immediately reflected by a shallow and frequent respiration accompanied by cyanosis. The tachypnea and cyanosis persisted for the rest of the experiment in group B (KCN only), whereas the symptoms
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were eliminated in group C (receiving treatment), suggesting an improvement in metabolism. Additionally, the rats experienced deterioration in brain metabolism with an immediate increase in interstitial lactate concentrations and L/P ratios once infusion of KCN commenced due to anaerobic glucose metabolism in the astrocytes (Figs. 3 and 6). This effect is in accordance with previous findings from animal experiments and human exposure to CN (7, 31). Pyruvate represents the point of entry to Krebs cycle, thus linking glycolysis to oxidative mitochondrial metabolism (38). In cases of hypoxia secondary to hypoperfusion, lactate dehydrogenase will convert pyruvate into lactate due to the lack of glucose supply (38). In the present experiments, however, the prolonged increase in L/P ratio (Fig. 6) after KCN administration seems to be a result of mitochondrial dysfunction rather than depleted nutrient supply for four reasons. First, the transient drop in MAP only lasted a few minutes, whereas the increase in L/P lasted for an extended period of time (t = 15–75 min). Second, MAP remained above 45–50 mmHg (Fig. 1), which may be considered as being above the lower level of CNS blood flow autoregulation in rats (35, 41). Third, CN infusion is known to cause cerebral hyperperfusion rather than hypoperfusion, thus rendering the surges in lactate and L/P ratios due to hyperperfusion even less likely (28). Finally, when glycolysis is triggered by hypoxia or ischemia, it normally implies a drop in PtO2. In the present experiment, the PtO2 was within normal range in the KCN-infused rats (Fig. 2), which emphasizes the blocking of the respiratory chain as the reason for the changes seen in interstitial microdialysate concentrations.

Treatment Effects

The CN molecules prevent the mitochondria from metabolizing glucose, thereby leaving the anaerobic metabolism as the only route of energy utilization. This is confirmed by the surges in glucose and lactate concentrations as well as the high L/P ratios observed in the interstitial brain tissue during the intoxication phase (Figs. 3, 4, and 6). In the KCN-exposed rats, the increase in interstitial glucose concentration is in line with previous studies showing that CN causes hyperglycemia and increases cerebral blood flow (1, 28). We found a less-pronounced glucose and lactate peak in the intoxicated rats receiving HBOT and OHcO (group C) compared with the KCN-group (group B) (Fig. 4). The attenuated surges and the subsequent decrease in glucose and lactate concentrations observed once OHcO and HBOT were administered indicate a beneficial effect. Moreover, the L/P ratio showed a downward trend (Fig. 6) once the treatment was initiated, suggesting that the combined treatments have an immediate effect toward restoring aerobic metabolism. Only the KCN-intoxicated rats without treatment experienced an increase in interstitial glycerol concentrations, whereas the rats receiving treatment had a distinct decrease in glycerol concentrations (Fig. 5). The results may reflect a less-pronounced degradation of the membrane phospholipids in the brain since glycerol constitutes a marker of tissue damage (38). This may be a positive prognostic sign. Breathing pure oxygen at pressures exceeding 500 kPa may induce cerebral vasoconstriction leading to reduced PtO2 (10). In this experiment, the rats were never exposed to such high pressures, and nothing indicates that cerebral blood flow was affected. In fact, PtO2 and glucose levels remained high during the HBOT. A systemic vasoconstriction may, however, explain the high MAP observed during the HBOT.

Mechanisms of Action

Fire victims are often exposed to a combination of carbon monoxide (CO) and CN. Both molecules bind to the mitochondrial cytochrome oxidase a3. In CO-intoxicated patients, HBOT attenuates long-term neurological sequelae (45, 48). Interestingly, in contrast with HBOT, normobaric oxygen breathing (inspired O2 fraction = 1.0) did not reduce mortality or neurological morbidity in similar experiments using microdialysis (5, 26, 27, 47). In addition, normobaric oxygen therapy had no effect on the reduced state of cytochrome oxidase a3 during CN exposures (12, 36, 49). Anecdotal evidence suggests that HBOT may improve morbidity and mortality in CN-poisoned humans (46). Although HBOT has been shown to improve mitochondrial oxidative processes during CN intoxication (43) and ameliorate CNS injuries both experimentally as well as in the clinical setting (11, 13, 44, 45, 48), when such injuries are induced by CO poisoning, normobaric oxygen breathing does not possess such capabilities.

It previously has been demonstrated that the treatment with either OHcO or HBOT reduced lactate and glucose concentrations in the first part of the experiment but did not find steady low concentrations during the latter part of the experiment (samples 10–21) (31). The rats treated with OHcO experienced respiratory distress and a low PtO2 throughout the experiment, whereas the rats treated only with HBOT showed a reappearance of cyanosis when the treatment ended. In keeping with this, our present results indicate that the combination therapy using OHcO and HBOT efficiently ameliorates lactate and glucose surges in the acute phase but also keeps a steady low interstitial concentration of lactate, glucose, and glycerol, as well as L/P ratio throughout the entire observation period. The rats receiving the combination therapy showed normal respiration and skin color throughout the experiment, as opposed to previous studies of therapy with either HBOT or OHcO (31).

The findings in this study suggest that the combination of OHcO and HBOT, administered immediately after acute KCN poisoning, ameliorates surges in interstitial brain lactate, glucose, and glycerol concentrations in the acute phase, while keeping a steady low interstitial brain lactate, glucose, and glycerol concentrations as well as low L/P ratios during the last part of the experiment. The low lactate concentrations and L/P ratios suggest an improved aerobic metabolism in OHcO and HBOT-treated rats. This effect is also seen during administration of HBOT in traumatic brain injuries (39, 40). The low glycerol concentrations in the intervention group indicate less cell damage, whereas the low glucose concentrations may be explained by restored glucose utilization in astrocytes and neurons. In addition, we found an improvement in respiration in combination with the disappearance of cyanosis and hemodynamic instability throughout the period of observation.

In conclusion, the present study indicates that OHcO and HBOT may have a beneficial, additive effect on cerebral metabolism in KCN-poisoned rats. The effect appears to be persistent in contrast with individual treatment regimes (31). Future studies are warranted to describe the mechanisms of
action of combined administration of OHCob and HBOT during CN poisoning.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.B.H., N.V.O., and O.H. conception and design of research; M.B.H. and O.H. performed experiments; M.B.H. and O.H. interpreted results of experiments; M.B.H. prepared figures; M.B.H., N.V.O., and O.H. drafted manuscript; M.B.H., N.V.O., and O.H. edited and revised manuscript; M.B.H., N.V.O., and O.H. approved final version of manuscript.

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