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Hyperbaric oxygen therapy or hydroxycobalamin attenuates surges in brain interstitial lactate and glucose; and hyperbaric oxygen improves respiratory status in cyanide-intoxicated rats

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ABSTRACT

Cyanide (CN) intoxication inhibits cellular oxidative metabolism and may result in brain damage. Hydroxycobalamin (OHCob) is one among other antidotes that may be used following intoxication with CN. Hyperbaric oxygen (HBO₂) is recommended when supportive measures or antidotes fail. However, the effect of hydroxycobalamin or HBO₂ on brain lactate and glucose concentrations during CN intoxication is unknown.

We used intracerebral microdialysis to study the *in vivo* effect of hydroxycobalamin or HBO₂ treatment on acute CN-induced deterioration in brain metabolism. Anesthetized rats were allocated to four groups receiving potassium CN (KCN) 5.4 mg/kg or vehicle intra-arterially: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO₂ (284 kPa for 90 minutes). KCN alone caused a prompt increase in interstitial brain lactate and glucose concentrations peaking at 60 minutes. Both hydroxycobalamin and HBO₂ abolished KCN-induced increases in brain lactate and glucose concentration. However, whereas HBO₂ treatment increased cerebral PtO₂ and reduced respiratory distress and cyanosis, OHCob did not have this beneficial effect. In conclusion, CN intoxication in anesthetized rats produces specific uncoupling of cerebral oxidative metabolism resulting in interstitial lactate and glucose surges that may be ameliorated by treatment with either hydroxycobalamin or HBO₂.

INTRODUCTION:

Cyanide (CN) is a potentially lethal intracellular poison [1]. The primary effect of CN is an uncoupling of the mitochondria respiration chain with subsequent depletion of adenosine triphosphate (ATP). This results in a universal hypoxia and an increase in lactate production resulting in metabolic acidosis [2]. Previous reports have shown that plasma lactate concentration correlates positively with blood CN concentration, and monitoring of plasma lactate may be useful in assessing severity of human CN poisoning [3]. CN poisoning is recognized as a cause of neurological disability, ranging in humans from various extrapyramidal syndromes to post-anoxic vegetative states [4]. CN binds to the ferric ion of cytochrome oxidase a₃, inducing a non-competitive inhibition of the mitochondria activity.

A binding of CN is most often referred to as being irreversible [2,5]. However, recent evidence suggests that

CN binding to cytochrome oxidase is reversible. Stubauer *et al.* [6] demonstrated the presence of competition between CN and nitric oxide (•NO) in the binding to cytochrome c oxidase. Moreover, high concentrations of •NO may decrease CN inhibition of cytochrome oxidase [7]. In this respect, it may be of importance that hyperbaric oxygen (HBO₂) treatment has been shown to alter •NO production by increasing its bioavailability *in vivo* [8-11]. In a recent report, we demonstrated that HBO₂ treatment was able to induce changes in whole-blood CN concentrations [12]. The results suggested that this effect was caused by a competitive mass displacement of CN from the intracellular compartment, although this mechanism *per se* remains unproven [12].

Hydroxycobalamin (OHCob) is a recommended antidote in the treatment of CN poisoning. OHCob acts by covalent binding to CN and forms cyanocobalamin, which is a B12 vitamin [13,14]. Given intravenously,

OHCob distributes to the erythrocytes and plasma cells, and after 30 minutes also reaches the cerebrospinal fluid [15]. OHCob mainly has its effect in the extracellular compartment [16]. Whether OHCob has any effect on brain cell metabolism as measured by changes in interstitial lactate and glucose concentrations during CN poisoning has not been investigated before.

The purpose of the present experiments was to study the effect of OHCob or HBO₂ treatments on cerebral cell metabolism by the use of a microdialysis model in rats, thereby measuring the *in vivo* changes in brain interstitial lactate and glucose concentrations during CN intoxication. We hypothesized that both OHCob and HBO₂ treatment may improve brain metabolism during acute CN intoxication.

METHODS

The animal experiments were approved by a government-granted license from the Danish Animals Ethical Committee. Female Sprague Dawley rats (250g) from Taconic (Denmark) were used. The animals were housed in a temperature- (22-24°C) and moisture-controlled (40-70%) room with a 12-hour light/dark cycle (light on from 6:00 a.m. to 6:00 p.m.). The rats were given water and feeding *ad libitum* using a standard rodent diet with 140-mmol/kg sodium, 275-mmol/kg potassium and 23% protein (Altromin International, Lage, Germany).

Animal preparation and experimental protocol

Rats were anesthetized with fentanyl 0.315 mg/ml + fluanizone 10 mg/ml (Hypnorm, Veta-Pharma™, UK) and midazolam (5 mg/ml). Start dose was 0.3 ml/100g by means of subcutaneous (s.c.) injection. Depth of anesthesia was maintained with fentanyl + fluanizone and midazolam 1.25 mg/250g given s.c. every 30 minutes.

The anesthetized rat was positioned on a heating platform with the head between the ear bars in a stereotaxic frame (Lab Standard™ Stereotaxicinstrument, Stoelting®). A thermocouple was placed in the vagina and connected to a CMA/150™ Temperature Controller thermostat maintaining the rat temperature at 37°C. With the rat in the stereotaxic frame, two bore holes were drilled, penetrating the cranial bone using a dental drill (Bravo Micromotor™, Danish Nordenta A/S, Hard metal round burs RA 1/008+1/009), initially leaving the dura intact. The two bore holes were placed 3 mm frontal of *fonticulus anterior major* and 3 mm to the right of the *sutura sagittalis* for the microdialysis catheter and 3 mm left of the *sutura sagittalis* for the catheter meas-

uring tissue oxygen partial pressure (P_tO₂) (Integra™, Licox® Oxygen probe Ref. CC1, Germany).

Once the microdialysis and P_tO₂ catheters were in place, the rats were removed from the stereotaxic frame and placed on the heating platform in the supine position. A polyethylene catheter, model TYGON™ (id. 0.035mm from Saint Gobain Plastics®), was then inserted in the right femoral artery. The catheter was forwarded 1-1.5 cm to make sure of the intraluminal placement for subsequent blood pressure registration and CN administration.

For rats allocated to HBO₂ therapy, a tracheal cannula was inserted (polyethylene tubing; ID 1.5 mm). Further, polyethylene tubing was placed subcutaneously on the back on the rat in the *regio lumbaris dexter* using blunt dissection. Once the rat was in the chamber, the polyethylene tube was attached to a chamber penetration allowing supplementary anesthesia to be given during the HBO₂ treatment. Before HBO₂ treatments with pressurization could be initiated, the tracheal cannula was connected to a T-shaped tube in the chamber breathing system (*see below*). The right femoral artery catheter, filled with isotonic saline, was then connected to a pressure transducer (Edwards Life Sciences™) placed inside the pressure chamber, which allowed for the continuous measurement of mean arterial blood pressure (MAP). The catheter was kept patent by a continuous infusion of non-heparin zed saline by means of a syringe pump (SAGE Instruments, model 341) at a rate of 1 ml/hour. A continuous real-time record of temperature and MAP was obtained on a PC via Picolog™ software. A thermocouple placed in the rectum was then connected to the oxygen partial pressure measuring unit (Licox CMP tissue oxygen pressure monitor by Integral Neuro-Sciences™) to ensure a constant thermocalibration of P_tO₂ measurements. The P_tO₂ catheter was auto-calibrated once the connections were made. The P_tO₂ and temperature measurements were noted every 15 minutes.

For rats allocated to HBO₂ treatments, the chamber was pressurized using air, with the rat breathing 100% oxygen through the tracheotomy connected to the chamber breathing system. The chamber was pressurized over two minutes to 284 kPa. The HBO₂ treatment lasted 90 minutes, after which time the chamber was decompressed to 101.3 kPa (*i.e.*, atmospheric pressure). Once at 101.3 kPa the chamber steel lid was removed and the rat's tracheal cannula disconnected from the chamber breathing system and the rat switched back to spontaneous air breathing.

Rats were allocated to four different experimental groups (N =number of rats):

1. Controls ($N=5$), vehicle isot. NaCl 1.0 ml intra-arterially (i.a.).
2. KCN 5.4 mg/kg intra-arterially poisoned rats ($N=10$). In this group lactate concentrations in whole blood were also measured.
3. KCN 5.4 mg/kg intra-arterially poisoned rats receiving hydroxycobalamin 100mg/kg ($N=9$)
4. KCN 5.4 mg/kg intra-arterially poisoned rats treated with HBO₂ at 285 kPa in 90 minutes ($N=11$)

In order to verify that the observed changes in microdialysate lactate measurements were correlated to the infusion of CN, two supplemental control experiments (*A* and *B*) were performed.

'A' control experiments

During simultaneous cerebral microdialysate sampling, four rats were exposed to a KCN injection of 5.4 mg/kg twice in the femoral artery catheter, with a 75-minute interval between the two injections. It was found that KCN given twice caused a reproducible increase in brain lactate dialysate, the second KCN injection at 75 minutes causing a significant increase in interstitial lactate concentration (0.94 ± 0.16 mmol/l) as compared to KCN at Sample 135-300 minutes in Group 2 rats (Sample 10-21: 0.27 ± 0.10 mmol/l), $P<0.0001$ by means of independent T-test. The control experiment indicated that femoral artery KCN infusion was indeed associated with an increase in cerebral lactate concentrations as verified in the microdialysate fluid.

'B' control experiments

Following KCN infusion, CN will bind universally to the tissue cells. Further, previous reports have demonstrated that lactate will pass the blood-brain barrier [17, 18]. Accordingly, an experiment was performed verifying that changes of lactate concentrations in cerebral dialysate were primarily caused by intracellular cerebral lactate formation, rather than by random fluctuations in blood lactate values and possible redistribution from peripheral tissues. By means of repeated experiments, a lactate dose of 0.06g Sodium L-lactate (Sigma-Aldrich Logistic[®] GmbH) dissolved in 0.9 ml saline and given intra-arterially, was found to produce a similar increase in blood lactate concentrations (mean blood lactate= 5.2 ± 3.2 mmol/l) as seen during the intra-arterial administration of 5.4 mg/kg KCN

(mean blood lactate= 5.6 ± 3.9 mmol/l) – (Figure 2, Page 226). When the mean blood lactate concentration following 0.06g Sodium L-lactate infusion is compared to the mean blood lactate concentration after 5.4 mg/kg KCN infusion, the difference was not significant ($P=0.84$) by means of independent T-test.

Subsequently, another five rats were exposed to an intra-arterial lactate dose of 0.06g Sodium L-lactate in which simultaneous cerebral microdialysis procedures were performed. The results were compared to Group 2 animals exposed to KCN poisoning as outlined above.

The Sodium L-lactate infusion of 0.06g produced a very short lasting peak in interstitial cerebral lactate concentrations, followed by an immediate decrease to normal lactate concentration within 30 minutes (mean lactate= 0.50 ± 0.08 mmol/l). However, when KCN is infused, cerebral lactate concentrations rise slowly and remain increased for more than 100 minutes (Figure 3, Page 227), reaching normal values at 225 minutes after KCN infusion (mean= 0.66 ± 0.13 mmol/l).

When the initial increase in interstitial mean lactate concentration after infusion of Sodium L-lactate is compared to that of KCN infusion (*i.e.*, Sample 3-9) the difference is significant ($P=0.0315$, by means of independent T-test). Accordingly, the infusion of KCN caused a quantitatively as well as a qualitatively different behavior of cerebral lactate concentrations as compared to lactate infusion in blood.

Although a certain level of peripheral influx of lactate cannot be excluded, we take this observation as evidence that the observed lactate concentrations measured by cerebral microdialysis during KCN poisoning are caused primarily by intracerebral metabolic changes rather than the influx of lactate from peripheral blood. This conclusion is strengthened by the fact that 5.4 mg/kg i.a. KCN, produce a plasma lactate concentration peaking at >10 mmol/l less than 30 minutes after infusion, with a fast decay to normal level at only 75 minutes (Figure 2). If lactate concentrations in cerebral microdialysis fluid were entirely dependent on extracerebral influx, one would expect to see not only a much faster increase but also a higher concentration level [19] as well as a faster decay in interstitial lactate microdialysate.

Pressurization system

Compression and decompression was performed in a specially designed pressure chamber (chamber volume 0.02m^3) with a horizontal viewing port in the lid [20]. A small fan (ventilator) placed in the bottom of the

FIGURE 2

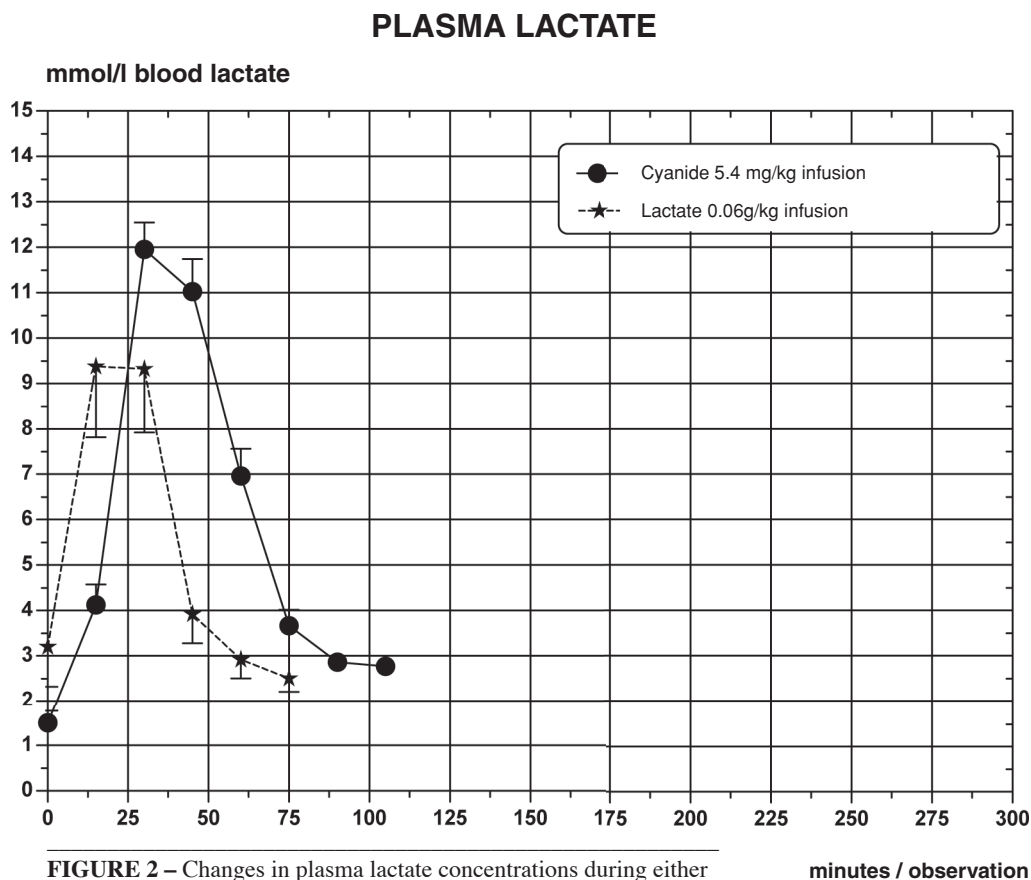


FIGURE 2 – Changes in plasma lactate concentrations during either intra-arterial KCN intoxication (5.4mg/kg) or intra-arterial lactate infusion (0.06g Sodium L-lactate from Sigma-Aldrich Logistic® GmbH).

chamber mixed the chamber atmosphere at frequent intervals. Penetrations were made for a chamber atmosphere heating system. The breathing mixture was supplied continuously in silicone tubing at a pressure slightly above chamber pressure, with a T-connection for the rat's tracheal cannula. The tube was connected to the exhaust outlet via a specially designed overboard dump valve (Ottestad Breathing Systems™, Norway).

The chamber was compressed on air as chamber gas only. The overboard dump valve was adjusted to maintain a maximum positive pressure corresponding to 1.2-1.6 cm H₂O above the chamber pressure. The rat's tracheal cannula was connected via the T-connection to the respiratory gas circuit, through which the breathing gas, *i.e.*, oxygen, free-flowed at 1500-2000 ml/minute, as verified by a flow meter. All rats remained unaffected with respect to blood pressure and ventilation when they were connected to the overboard dump valve system.

Intracerebral microdialysis

In this report, concentrations of lactate and glucose were measured in the brain interstitium by inserting a CMA® microdialysis probe 1 mm into the *crus of corpus callosum* of the rat brain, while the rat was fixed in a stereotaxic device as outlined above. The CMA® microdialysis guide cannula was implanted in the *crus of corpus callosum* as it has been shown that the *corpus callosum* shows considerably increased oxidative enzyme activity after CN injection [21]. The guide cannula penetrating the skull was kept in place with dental zinc phosphate cement (Poscal™, VOCO GmbH) attached to two screws on each side. Stainless steel dummy blockers were inserted into the guide cannula and fixed until insertion of the microdialysis probe (CMA12™ micro-dialysis probe with 4-mm tip length, 500µm outer diameter). CMA Perfusion Fluid CNS consisting of 147 mmol/L NaCl, 2.7 mmol/l KCL, 1.2 mmol/l CaCl₂

FIGURE 3

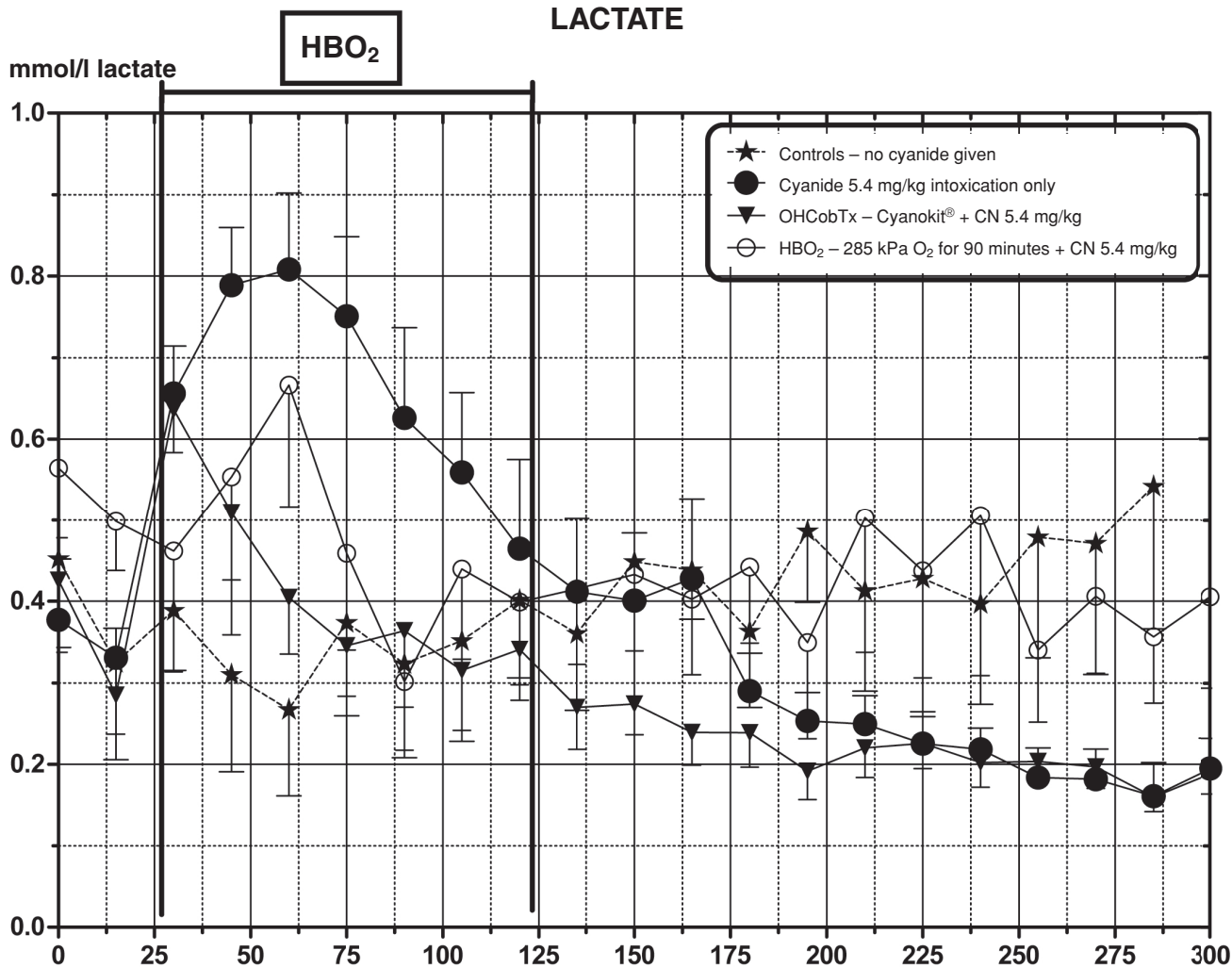


FIGURE 3 – Changes in interstitial cerebral lactate concentrations during either: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO₂ (284 kPa for 90 minutes). Horizontal bar and square box showing time of 90 minutes' HBO₂ treatment. Mean values are given \pm SEM.

and 0.85 mmol/L MgCl₂ was infused through the microdialysis probe using a CMA/100™ microinjection pump (CMA/microdialysis™ AB, Stockholm, Sweden).

Once the rat was placed inside the pressure chamber, the inlet of the microdialysis probe was connected through fluorinated ethylene propylene (FEP) tubing to the CMA 402™ syringe pump, which was placed outside the chamber. The microdialysis probes outlet was then connected through FEP tubing (diameter 120µm) to the microdialysis sampler (CMA/140 micro fraction collector, Carnegie Medicine AB, Stockholm, Sweden), which was placed inside the pressure chamber. The CMA/100

microinjection pump was mounted with a 1.0-ml syringe containing the artificial CNS perfusion fluid (*see above*) and the pump preset to an infusion rate of 2 µl/minute. Samples of cerebral interstitial fluid were collected at every 15 minutes, giving a sample volume of 30µl.

The samples were stored at 4°C for analysis on the following day, or at minus 20°C if analysis was to be performed at a later stage. The cerebrospinal fluid was analyzed by a CMA600™ Microdialysis Analyzer. First sample was collected at time $t=0$ minutes and before intervention with infusion of either vehicle (isotonic saline) in control animals or KCN (all other groups).

At 15 minutes the rat was given 5.4 mg/kg KCN depending on allocated group. When KCN was infused, the catheter was flushed with 0.3 ml saline to ensure no CN remained in the femoral artery catheter and all CN was distributed into the bloodstream. The method of i.a. KCN administration has been described in a previous report [22]. Treatment intervention commenced after 30 minutes with either OHCob or HBO₂ depending on treatment group allocation. When the last microdialysis sample was collected, the rat was sacrificed by injection of thiomebumal through the catheter in the right femoral artery

Once the rat was placed inside the chamber, the inlet of the microdialysis probe was connected through FEP tubing to the CMA 402™ syringe pump, which was placed outside the chamber. The microdialysis probes outlet was then connected through FEP tubing (diameter 120µm) to the microdialysis sampler (CMA/140 micro fraction collector, Carnegie Medicine AB, Stockholm, Sweden), which was placed inside the pressure chamber. The CMA/100 microinjection pump was mounted with a 1.0-ml syringe, containing the artificial CNS perfusion fluid (*see above*) and the pump preset to an infusion rate of 2 µl/minute. Samples of cerebral interstitial fluid were collected at every 15 minutes, giving a sample volume of 30µl. The samples were stored at 4°C for analysis on the following day or at minus 20°C if analysis was to be performed at a later stage.

The cerebrospinal fluid was analyzed by a CMA600™ Microdialysis Analyzer. The first sample was collected at time $t=0$ minutes and before intervention with infusion of either vehicle (isotonic saline) in control animals or KCN (all other groups). At 15 minutes the rat was given 5.4mg/kg KCN depending on the allocated group. When KCN was infused, the catheter was flushed with 0.3 ml saline to ensure no CN remained in the femoral artery catheter and all CN was distributed into the bloodstream. The method of intra-arterial KCN administration has been described in a previous report [22]. Treatment intervention commenced after 30 minutes with either OHCob or HBO₂ depending on treatment group allocation. When the last microdialysis sample was collected, the rat was sacrificed by injection of thiomebumal through the catheter in the right femoral artery.

Data analysis and statistics

Mean values of lactate and glucose concentrations in brain interstitial microdialysate fluid were calculated for every 15 minutes during the observation period, giving a total of 21 data sets. Each experimental group of

data sets were then subdivided into two groups, with the first one representing the time of observation during HBO₂ treatments, *i.e.*, observation samples from Number 3-9. The other data set represents the time of observations after HBO₂ treatment until termination of the experiment, *i.e.*, samples from Number 10-21.

To examine whether the difference between two data sets of mean values of lactate or glucose concentrations were different from zero, test for normality by means of Kolmogorov and Smirnov (KS) test followed by analysis of variance (ANOVA) was performed on the difference between mean values in the different treatment groups [23,24]. The difference between mean values of the various treatment groups was then analyzed by use of the Student-Newman Keuls procedure for multiple comparisons of means between groups using the SSPS statistical computer software [23,24]. For all comparisons, $P<0.05$ is regarded as the criteria for significance.

RESULTS

General symptoms

Control experiments: Anesthetized rats equipped with cerebral microdialysis and P_iO₂ catheters remained unaffected with respect to MAP and respiration during control experiments. MAP was in the range of 80-100 mmHg throughout the observation period.

CN poisoning: During administration of CN, the immediate effect was general seizures that lasted about 10 seconds, followed by respiratory arrest with a simultaneous fall in MAP from 80-100 mmHg to 40-50 mmHg. After 30-60 seconds, the respiration returned followed by shallow and frequent respiration and signs of peripheral cyanosis for the rest of the experiment. The return of respiration was followed by a rise in MAP to 80-90 mmHg as the most frequent interval. This lasted during the remainder of the experiment.

OHCob: During administration of OHCob in the CN-intoxicated rat, the immediate effect was seizures located in the ipsilateral leg of the right femoral artery infusion. These seizures lasted a few seconds, after which no further seizures were observed in the observation period. A reddening of the skin and urine was observed in all rats given OHCob treatment, which remained visible throughout the observation period. Animals given OHCob continued to show signs of shallow and frequent respiration, whereas cyanosis was not present due to the reddening of the skin. MAP was in the range of 110-125 mmHg during the entire observation period.

FIGURE 1

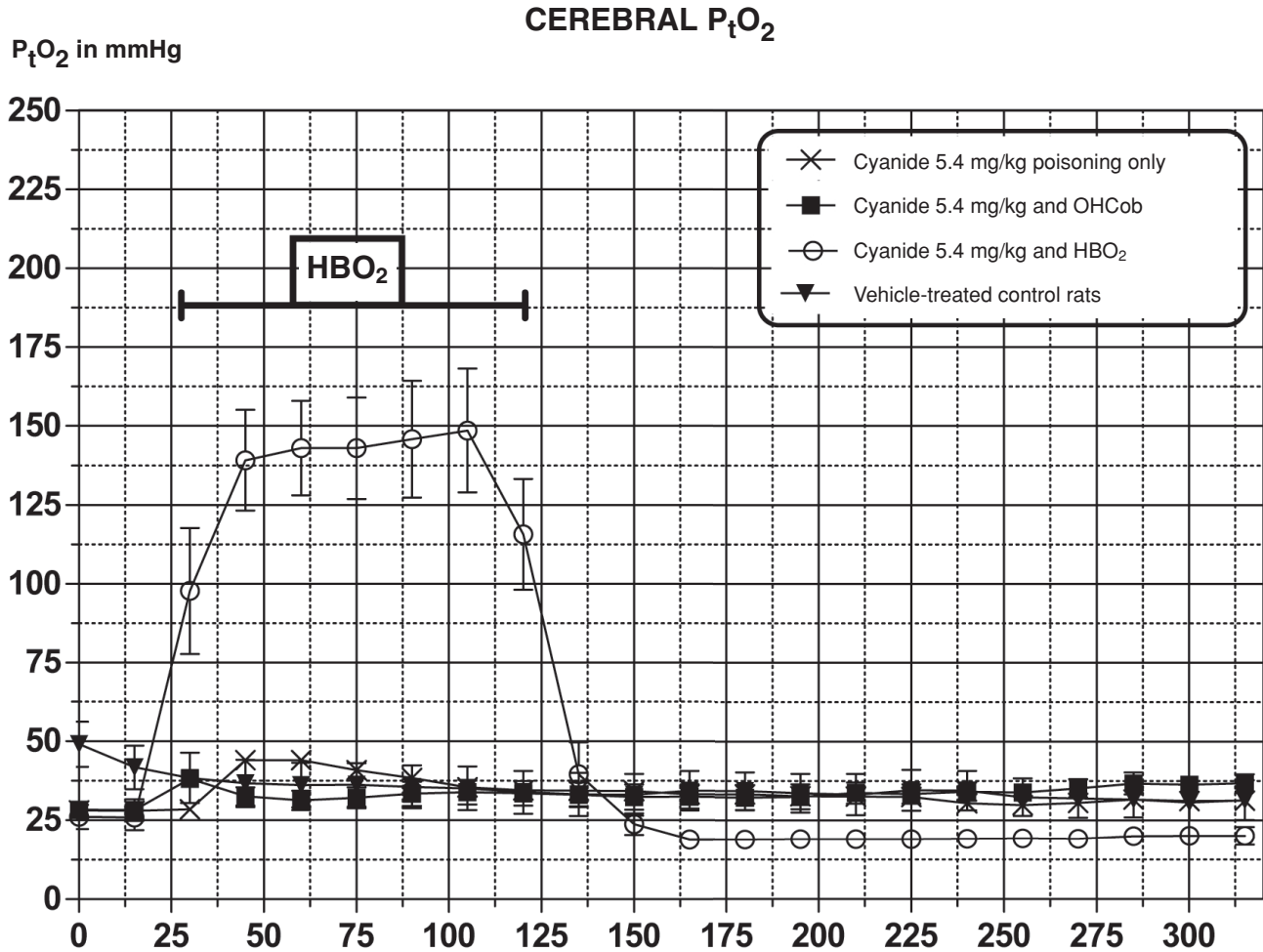


FIGURE 1 – P_tO_2 measurements in rat brain during either: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO₂ (284 kPa for 90 minutes). Horizontal bar and square box showing time of 90 minutes' HBO₂ treatment. Mean values are given \pm SEM.

HBO₂: CN-intoxicated rats with respiratory distress and cyanosis which were subsequently given HBO₂ treatment showed a fast improvement in respiration and disappearance of cyanosis. Once HBO₂ treatment subsided, the rats remained free of respiratory distress and cyanosis for the following one-hour observation time. Thereafter, the cyanosis was observed to reappear and lasted for the rest of the experiment. During HBO₂ treatments, MAP would rise from 100-135 mmHg to 140-150 mmHg, as the most frequent interval and remained at this level. After HBO₂ treatment, the MAP would stabilize at 100-135 mmHg and remain there throughout the observation period.

Measurements of cerebral P_tO_2

Values recorded from P_tO_2 measurements are shown in Figure 1 (above). After initiation of HBO₂, a major rise in P_tO_2 of about 70-100 mmHg to an average of 140 mmHg was observed. The tissue hyperoxia remained stable until the HBO₂ therapy was ceased and from which point the P_tO_2 returned to pre-HBO₂ level at 35 mmHg and remained there for the rest of the observation period (Figure 1). P_tO_2 in the other series remained unchanged (Figure 1).

TABLE 1

* Values given \pm SD

Treatment groups (N=number of rats) Time	LACTATE		GLUCOSE	
	Mean interstitial cerebral lactate concentration in mmol/l *		Mean interstitial cerebral glucose concentration in mmol/l *	
	values given \pm SD		values given \pm SD	
	Sample 3-9 30-120 min	Sample 10-21 135-300 min	Sample 3-9 30-120 min	Sample 10-21 135-300 min
Control (N=5)	0.34 \pm 0.05 ¹	0.44 \pm 0.05	0.25 \pm 0.07	0.31 \pm 0.03 ⁷
KCN (N=10)	0.66 \pm 0.13 ²	0.27 \pm 0.10 ³	0.31 \pm 0.12	0.25 \pm 0.11
KCN + OHCob (N=9)	0.42 \pm 0.12	0.22 \pm 0.03 ⁴	0.13 \pm 0.05 ⁵	0.07 \pm 0.02 ⁸
KCN + HBO ₂ (N=11)	0.47 \pm 0.12	0.42 \pm 0.05	0.16 \pm 0.03 ⁶	0.17 \pm 0.04 ⁹

¹ Controls vs KCN $P < 0.001$. ² KCN vs OHCob and HBO₂; $P < 0.001$ and $P < 0.01$ respectively. ³ KCN vs Control and HBO₂; $P < 0.001$ and $P < 0.001$ respectively. ⁴ OHCob vs Control and HBO₂; $P < 0.001$ and $P < 0.001$ respectively.

⁵ OHCob vs Controls and KCN; $P < 0.01$ and $P < 0.001$ respectively. ⁶ HBO₂ vs Controls and KCN; $P < 0.05$ and $P < 0.01$ respectively. ⁷ Controls vs KCN; $P < 0.05$. ⁸ OHCob vs Controls, KCN and HBO₂; $v < 0.001$ for all comparisons.

⁹ HBO₂ vs Control and KCN; $P < 0.001$ and $P < 0.01$ respectively.

Measurements of lactate and glucose concentrations in brain dialysate

Mean interstitial cerebral lactate and glucose concentrations are shown in Table 1 (above).

Control group: In rats allocated to the control group (N=5), continuous microdialysis demonstrated a steady lactate concentration ranging from 0.26-0.54 mmol/l (Figure 3). From initial baseline values ranging from 0.16-0.20 mmol/l during the first 60 minutes, glucose showed a slow increase with subsequent stabilization from between 0.28-0.35 mmol/L during the rest of the observation period (Figure 4, facing page).

Effects of CN infusion: Rats given CN infusion (N=10) showed a steady increase in lactate concentration reaching maximum values at 0.80 mmol/l 60 minutes from the first sample at $t=0$ (Figure 3). From here on, a continuous decrease was seen throughout the observation period and reaching a final average lactate concentration of 0.20 mmol/l at 300 minutes (Figure 3). The interstitial concentration of glucose showed a steady increase reaching a maximum at 0.42 mmol/l at 120 minutes into the observation period, from which point the glucose concentration steadily dropped toward 0.14 mmol/l at $t=300$ minutes (Figure 4).

Effects of OHCob after CN infusion: Rats being CN-poisoned and given OHCob at $t=15$ minutes (N=9) showed an initial steep increase in lactate concentration with a peak maximum at 0.64 mmol/l (Figure 3).

However, once infusion of OHCob at $t=30$ minutes (or data point 3 in Figure 3) commenced, lactate concentration immediately started to decrease toward control values with a continued decreasing tendency throughout the observation period (Figure 3). After OHCob infusion, glucose concentration remained low and within the range of 0.04-0.17 mmol/l throughout the observation period (Figure 4).

Effects of HBO₂ after CN infusion: The animals given CN i.a. at $t=15$ minutes and then treated with HBO₂ initiated at $t=30$ minutes (N=11) showed an initial increase in lactate concentration with a peak value of 0.67 mmol/l at $t=60$ minutes. After this point the lactate concentration would subsequently decrease, reaching its lowest concentration at $t=90$ minutes. From here on, the lactate concentration in the post-HBO₂ observation period increased slowly (Figure 3). During and after HBO₂ treatment, glucose concentration remained stable within the range of 0.13-0.25 mmol/l (Figure 4).

Comparison of brain dialysate after treatment with OHCob or HBO₂

Lactate: In Sample 3-9 the interstitial lactate concentration was smaller in control rats as compared with rats exposed to KCN intoxication ($P < 0.001$). In Sample 3-9, the control rats vs. rats treated with either OHCob or HBO₂ were not different from each other ($P > 0.05$).

FIGURE 4

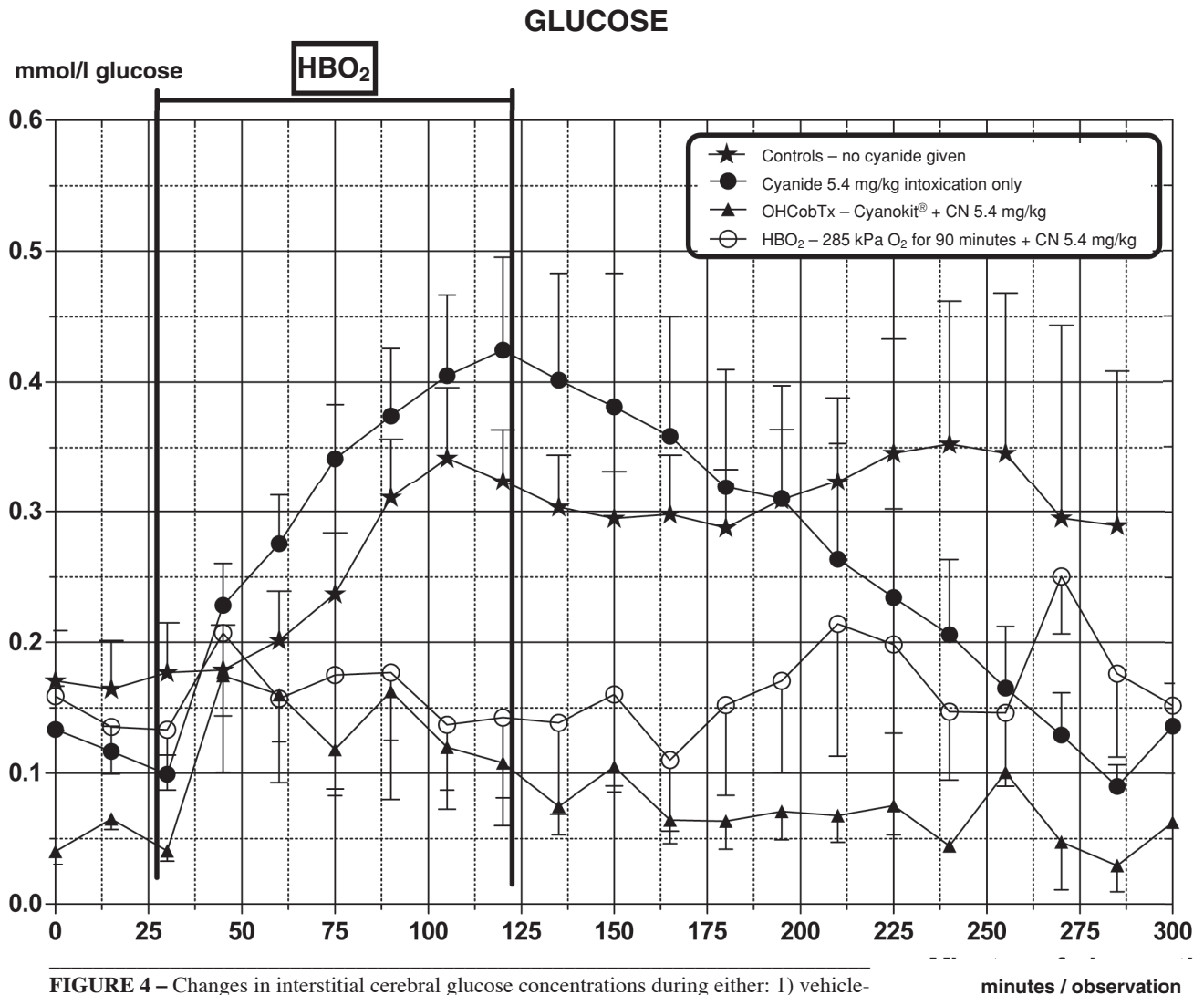


FIGURE 4 – Changes in interstitial cerebral glucose concentrations during either: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO₂ (284 kPa for 90 minutes). Horizontal bar and square box showing time of 90 minutes HBO₂ treatment. Mean values are given ± SEM.

However, in rats treated with either OHCob or HBO₂ the lactate concentrations in Sample 3-9 were significantly lower as compared to KCN-exposed rats ($P < 0.001$ and $P < 0.01$ respectively). When the mean lactate concentrations of Sample 3-9 were compared, there were no significant differences between OHCob- and HBO₂-treated rats ($P > 0.05$).

In Sample 10-21 interstitial lactate concentrations which had remained stable throughout the observation period in control rats not exposed to KCN intoxication remained at their mean level of 0.44 mmol/l lactate, whereas the mean lactate concentration of KCN-exposed

rats dropped significantly below the control level ($P < 0.001$). In rats treated with OHCob the mean lactate concentration during Sample 10-21 was significantly lower as compared to control ($P < 0.001$) as well as to HBO₂-treated animals ($P < 0.001$). OHCob-treated rats were not different from KCN-exposed rats during Sample 10-21 ($P > 0.05$). With respect to HBO₂-treated animals there was no difference in lactate concentrations when compared to control rats ($P > 0.05$), but there was significantly greater lactate concentrations in HBO₂-treated rats as compared to KCN-treated rats ($P < 0.001$).

Glucose: In Sample 3-9, there were no differences in interstitial glucose concentrations between control rats as compared to rats given KCN ($P>0.05$). Rats treated with either OHCob or HBO₂ were significantly lower in glucose concentrations as compared to both controls ($P<0.01$ and $P<0.05$, respectively) and KCN-intoxicated rats ($P<0.001$ and $P<0.01$, respectively). Glucose concentrations in OHCob and HBO₂-treated rats did not differ ($P>0.05$).

In Sample 10-21 control rats were significantly higher in glucose concentrations as compared with both KCN-exposed rats ($P<0.05$) and OHCob and HBO₂-treated rats ($P<0.001$ and $P<0.001$, respectively). Rats exposed to KCN only showed a significantly higher glucose concentration as compared to both OHCob and HBO₂-treated rats ($P<0.001$ and $P<0.01$). Furthermore, OHCob-treated rats were even lower in glucose concentrations in Sample 10-21 as compared with HBO₂-treated rats ($P<0.001$).

DISCUSSION

The primary effect of CN is a blocking of the mitochondrial respiration chain and stopping the formation of ATP. The result is universal hypoxia and metabolic acidosis caused by increased lactate levels [2]. In this report we studied the effects of OHCob and HBO₂ treatment on KCN-intoxicated rat brain by means of microdialysis measuring the changes in interstitial cerebral lactate and glucose concentrations.

It was found that in rats exposed to 5.4 mg/kg KCN infusion, an increase in interstitial cerebral as well as blood lactate concentrations was observed, together with symptoms such as seizures and respiratory distress. These symptoms are also observed in humans exposed to severe CN poisoning [1,25,26]. In the present study, CN was infused into the bloodstream of the femoral artery, as described in a previous report [12]. Since CN is a small lipid-soluble molecule and mainly undissociated, distribution and penetration of CN into tissue cells – especially tissues with a high blood perfusion rate such as the heart, central nervous system, liver, kidneys, skeletal muscle and spleen – are rapid [19]. Although CN will bind to the muscle tissue when given intra-arterially, the capacity of muscle tissue to act as a sink for CN is small; or it may release CN more readily, or metabolize CN via the enzyme rhodanese, than other organs [27].

In keeping with the control experiments using lactate infusion as described above, prolonged influx of CN from peripheral tissue compartments into the brain does not seem likely in these experiments. This is also confirmed

by the consistent decay observed in whole-blood CN concentrations seen during intra-arterial CN administration when compared to intraperitoneally CN-intoxicated animals, as demonstrated in our previous report [12]. At high pressures exceeding 303 kPa, pure oxygen breathing has been demonstrated to induce vasoconstriction, which may cause the reduction of cerebral P_tO₂ in certain regions of the rat brain [28,29]. This HBO₂-induced vasoconstriction may in part explain the observed higher MAP in the latter part of the observation phase as compared to controls and KCN-intoxicated rats. However, the fact that P_tO₂ increased significantly during HBO₂ treatments at 285 kPa in these experiments (*Figure 1*) suggests that regional cerebral blood flow was not affected to a degree causing reduced P_tO₂.

In control rats not exposed to CN intoxication, interstitial lactate concentration was within the normal range [30,31] throughout the observation period. However, interstitial glucose showed an increase with subsequent stabilization. Since control rats were not exposed to metabolic blockade by CN, the greater interstitial glucose concentration may partly be explained by an effect of anesthesia as demonstrated by Johansen *et al.* 1994 [32], who found an increase in plasma glucose during fluanizone (Hypnorm) anesthesia in rats. The mechanism may possibly be related to the strong opioid component (fentanyl) of fluanizone – an effect which may be reversed by HBO₂ [33,34]. Whether some other mechanism – such as cerebral blood flow changes, which may affect brain glucose metabolism (*see below*) – is involved in these experiments remains to be investigated.

The increase in brain interstitial as well as blood lactate concentrations during acute CN intoxication is in agreement with previous findings from experiments in animals and from human exposures to CN [2,35]. In rats exposed to the combined effect of anesthesia and CN intoxication, the increase observed in interstitial glucose concentration (*Figure 4*) is in agreement with observations by Akyildiz BN *et al.* and Yessoufou A *et al.* [36,37], who found that exposure to CN causes hyperglycemia in both humans and rats. Further, reports have shown that CN intoxication will cause an increase in cerebral blood flow [38,39]. An increase in regional cerebral blood flow correlates with increased interstitial glucose concentrations as demonstrated by Frykholm P *et al.* [40]. Frykholm P and co-workers found that changes in interstitial cerebral lactate concentration correlate to the cerebral metabolic rate of oxygen consumption, whereas glucose is more closely related to regional

cerebral blood flow changes. The fact that the glucose concentration decreases in the later phase of the observation – in spite of the high dose of CN – suggests that not all cerebral cells are blocked by CN, thus allowing for a continued glucose metabolism of the brain [41].

OHCob given intravenously will react with any CN present in blood and creates cyanocobalamin (vitamin B12), a non-toxic substance that is excreted via the kidneys [42]. Previous reports have suggested that OHCob has its primary effect in the extracellular compartment and that OHCob does not pass the blood-brain barrier [16]. However, others have found that OHCob does penetrate into the cerebrospinal fluid of the central nervous system with a time delay of approximately 30 minutes [15]. Since intra-arterially administered OHCob were able to ameliorate the CN-induced interstitial cerebral surge in lactate, the present results suggest that OHCob has an immediate effect on extravascular brain cell parenchyma during CN intoxication.

To our knowledge, this effect of OHCob on extravascular tissue cells in the brain has not been demonstrated before. If OHCob is able to pass the blood-brain barrier to reach the cell parenchyma of the brain, the capture of CN by OHCob should restore metabolic glucose utilization, which will reduce the interstitial glucose concentration as compared to controls. In these experiments, rats not exposed to CN intoxication had a higher interstitial glucose concentration as compared to OHCob-treated rats (*Figure 4*). However, OHCob given intravenously will increase cerebral blood flow [43], which should increase the cerebral glucose concentration. The mechanism may be linked to the scavenging of •NO by OHCob [44] caused by the interference of OHCob with the endothelial •NO synthase system. The effects of increased blood flow and scavenging of the potent vasodilator of •NO could partly explain the observation of a higher MAP in OHCob-treated rats during the latter part of the observation phase as compared to controls and KCN-intoxicated rats. Since OHCob does not affect the concentration of glucose in plasma directly [45], it seems justified to assume that the reduction in interstitial cerebral glucose concentration observed in these experiments is caused by an intracellular effect of OHCob.

Several reports have indicated that normobaric oxygen breathing has no effect on the reduced state of cytochrome oxidase a₃ during CN binding [46-48]. Because the cellular utilization of oxygen is impaired during CN poisoning and the rhodanese enzyme reaction seems insensitive to oxygen [49], it has been stated

that the effect of supplemental HBO₂ is uncertain. Also the synergistic effect of HBO₂ and the antidote sodium thiosulfate was no greater than sodium thiosulfate infusion combined with normobaric oxygen breathing [50]. By measuring reduced pyridine nucleotide in the renal cortex of rabbits, HBO₂, but not normobaric oxygen, was shown to improve mitochondrial oxidative processes during CN poisoning [51]. Irrespective of the contradictory results, HBO₂ has been shown to improve survival and improve tissue oxygenation in the clinical as well as in the experimental settings [52] and HBO₂ is recommended especially when supportive measures and other CN antidotes fail [50,53,54]. Furthermore, we recently demonstrated that HBO₂ treatment induced changes in whole-blood CN concentrations [12]. This effect may be caused by a competitive mass displacement of CN from the intracellular compartment, although that mechanism remains unproven [12]. In the cell, CN binds to the enzyme cytochrome oxidase a₃ (*i.e.*, complex IV in the mitochondrial electron transport chain) similar to carbon monoxide (CO) [55], thus blocking the mitochondrial respiration chain with subsequent depletion of adenosine triphosphate (ATP).

In addition, HBO₂, but not normobaric oxygen therapy, has been shown to be beneficial in ameliorating pathological events associated with central nervous system injuries in experimental as well as clinical studies on CO poisoning [56-60]. However, whereas CN binding to cytochrome oxidase appears to be independent of the oxygen tension, there seems to be a competition between CN and •NO. Although previous reports have suggested that cytochrome oxidase may act as a •NO reductase [6,61], Stubauer *et al.* [6] found no evidence for this proposed activity but rather established a competition between CN and •NO binding to cytochrome oxidase. High concentrations of •NO have been found to attenuate the inhibition of cytochrome oxidase induced by CN and CO [7,62]. In keeping with this, HBO₂ therapy, but not normobaric oxygen breathing, has been shown to increase the bioavailability of •NO [8-11,63]. These observations may explain the amelioration of the changes in interstitial cerebral lactate concentrations in the present experiments, but also provide a possible mechanism for the previously observed changes in whole-blood CN concentrations after HBO₂ therapy [12].

In the present results we observed that HBO₂ therapy reduced interstitial glucose concentration as opposed to controls and CN-exposed animals. If CN is displaced from cytochrome oxidase, brain metabolism – and thus

glucose utilization – may be restored according to the reasoning given above. However, whereas changes in cerebral lactate concentrations correlate to the oxygen consumption rate (36), brain glucose metabolism is complex and may be affected in a number of different ways. Firstly, reports have shown that HBO₂ reduces the glucose concentration in blood of diabetic rats [34] as well as in humans [33]. Whether this effect is seen in the non-diabetic organism has not been studied. Secondly, as discussed above, CN increases cerebral blood flow [34, 35] and causes hyperglycemia [32,33]. At the same time HBO₂ may induce cerebral blood flow reduction [26,27,42,43] partly explaining the reduction in interstitial glucose as compared to both KCN-intoxicated rats as well as to controls (*Table 1*). In the latter phase of the observation, the OHCob-treated rats displayed a significantly lower glucose concentration in comparison with HBO₂ (*Figure 4*). This seemingly protracted increase in glucose concentration after HBO₂, in comparison to OHCob, may reflect a continued CN intoxication once HBO₂ treatment has ceased, since HBO₂ does not bind CN as does OHCob.

Previously, glucose was regarded as the only energy substrate for neurons. However, reports have shown that lactate can be used as an energy substrate by neurons [64,65], even when ATP stores are depleted [66]. Lactate administration directly to the brain after reperfusion injury can effectively protect against ischemia-induced cell death and disability [66]. In patients, lactate administration may improve neurological outcome after traumatic brain injury [67]. However, lactate concentrations that remain elevated in cerebrospinal fluid as blood levels have normalized are associated with death and poor outcomes [68]; and since the correlation between the severity of CN intoxication and plasma lactate levels are well established [2,3],

treatment modalities in CN poisoning that aim to normalize cellular metabolism as well as interstitial and plasma lactate concentrations are indicated.

CONCLUSIONS

The present results suggest that HBO₂ treatment administered immediately after CN intoxication ameliorates the interstitial surge seen in cerebral lactate and glucose concentrations during treatment. The effect of HBO₂ on cerebral lactate and glucose concentrations are almost as effective as OHCob, although OHCob was more effective in reducing lactate as well as maintain low levels of glucose in the latter part of the observation phase. Furthermore, the effect of OHCob suggests that this antidote may act as a CN scavenger in the intracellular compartment as well. However, whereas HBO₂ treatment increased cerebral P_iO₂ and reduced respiratory distress and cyanosis, OHCob did not have this beneficial effect. Since HBO₂ is not a CN scavenger, the effect of HBO₂ may be mediated through •NO displacing CN from cytochrome oxidase. Once CN is displaced from the intracellular compartment, a subsequent increase in whole-blood CN concentrations may be seen. This could cause a rebound effect, with CN re-intoxication of the cells when CN diffuses back into the cells once HBO₂ treatment has subsided. Accordingly, further studies on the combined effect of HBO₂ and OHCob treatment are warranted.

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