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

Article *in* Undersea & hyperbaric medicine: journal of the Undersea and Hyperbaric Medical Society, Inc · July 2011

Source: PubMed

CITATIONS
9

READS
24

3 authors:

Pia Lawson-Smith
Rigshospitalet
4 PUBLICATIONS **81** CITATIONS

SEE PROFILE

Niels Vidiendal Olsen
University of Copenhagen
166 PUBLICATIONS **3,616** CITATIONS

SEE PROFILE

Ole Hyldegaard
Rigshospitalet
73 PUBLICATIONS **446** CITATIONS

SEE PROFILE

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

LAWSON-SMITH P.[1], OLSEN N.V.[2,3], HYLDEGAARD O.[1]

[1] Laboratory of Hyperbaric Medicine, Department of Anesthesia, Centre of Head and Orthopedics, Copenhagen University Hospital (Rigshospitalet), Denmark;

[2] Department of Neuroanaesthesia, The Neuroscience Center, Copenhagen University Hospital, (Rigshospitalet), Denmark

[3] Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark

CORRESPONDING AUTHOR: Dr. Pia Lawson-Smith – *lawson_smith@dadlnet.dk*

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 (HBO_2) is recommended when supportive measures or antidotes fail. However, the effect of hydroxycobalamin or HBO_2 on brain lactate and glucose concentrations during CN intoxication is unknown.

We used intracerebral microdialysis to study the *in vivo* effect of hydroxycobalamin or HBO_2 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 HBO_2 (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 HBO_2 abolished KCN-induced increases in brain lactate and glucose concentration. However, whereas HBO_2 treatment increased cerebral PtO_2 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 HBO_2.

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 a,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 (HBO_2) treatment has been shown to alter •NO production by increasing its bioavailability *in vivo* [8-11]. In a recent report, we demonstrated that HBO_2 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 HBO_2 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 HBO_2 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 measuring tissue oxygen partial pressure (P_tO_2) (Integra™, Licox® Oxygen probe Ref. CC1, Germany).

Once the microdialysis and P_tO_2 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 HBO_2 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 HBO_2 treatment. Before HBO_2 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_tO_2 measurements. The P_tO_2 catheter was autocalibrated once the connections were made. The P_tO_2 and temperature measurements were noted every 15 minutes.

For rats allocated to HBO_2 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 HBO_2 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 HBO_2 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 0.06 g 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 0.06 g 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 micro-dialysis 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

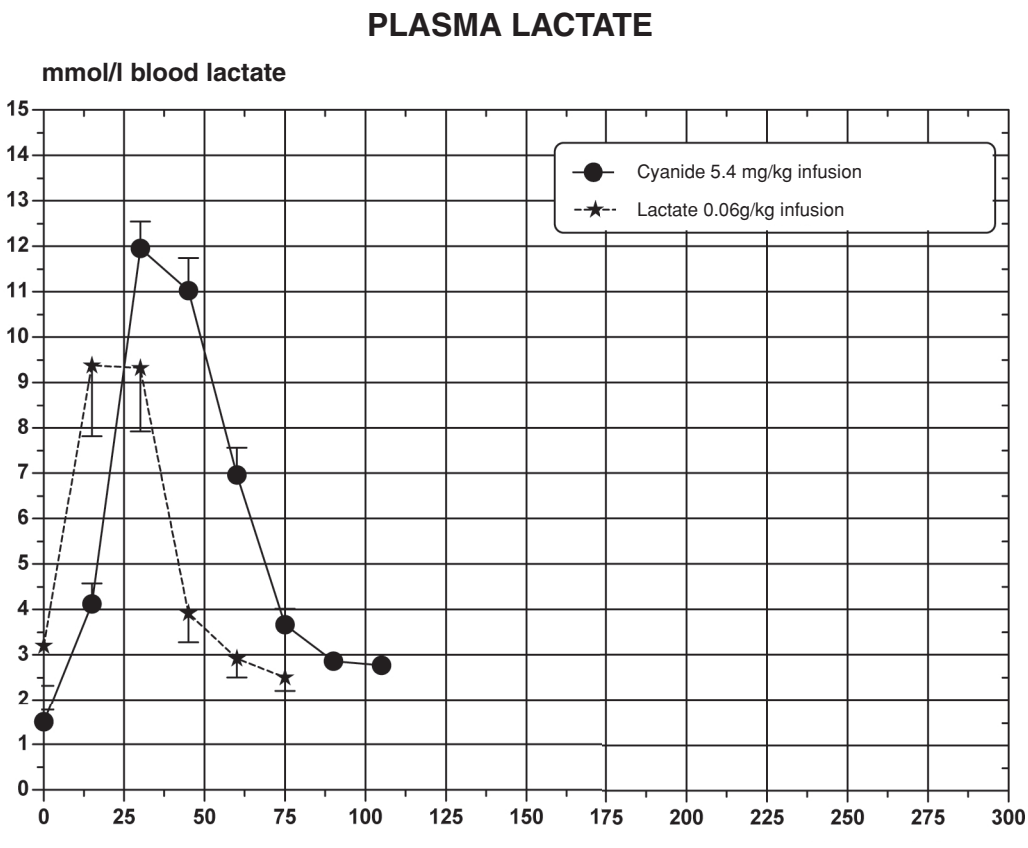

FIGURE 2 – Changes in plasma lactate concentrations during either intra-arterial KCN intoxication (5.4mg/kg) or intra-arterial lactate infusion (0.06g Sodium L-lactate from Sigma-Aldrich Logistic® GmbH).

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 H_2O 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 $CaCl_2$

FIGURE 3

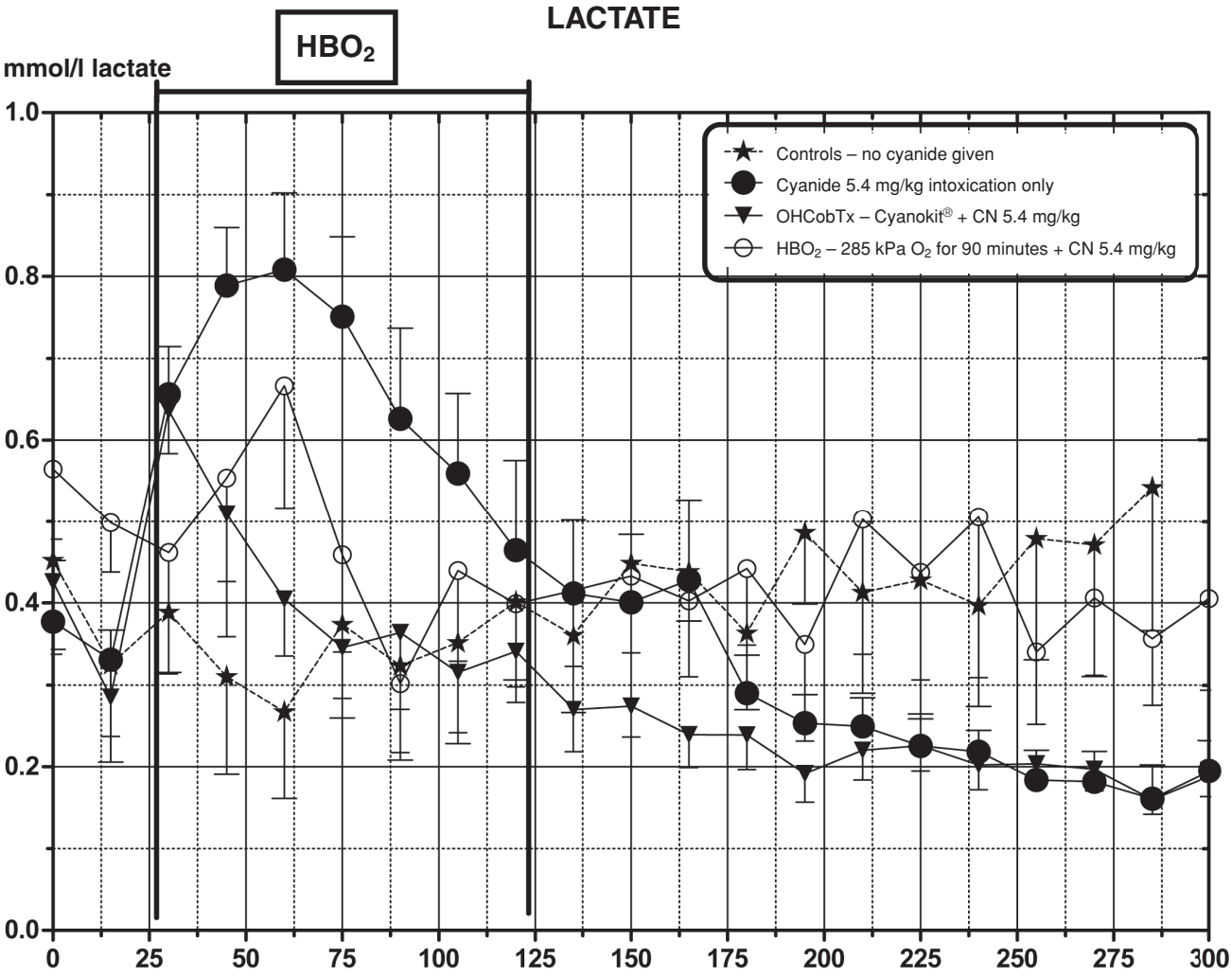

FIGURE 3 – Changes in interstitial cerebral lactate concentrations during either: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO_2 (284 kPa for 90 minutes). Horizontal bar and square box showing time of 90 minutes' HBO_2 treatment. Mean values are given ± SEM.

and 0.85 mmol/L $MgCl_2$ 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 4ºC for analysis on the following day, or at minus 20ºC 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 HBO_2 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 4ºC for analysis on the following day or at minus 20ºC 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 HBO_2 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 HBO_2 treatments, *i.e.*, observation samples from Number 3-9. The other data set represents the time of observations after HBO_2 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_tO_2 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

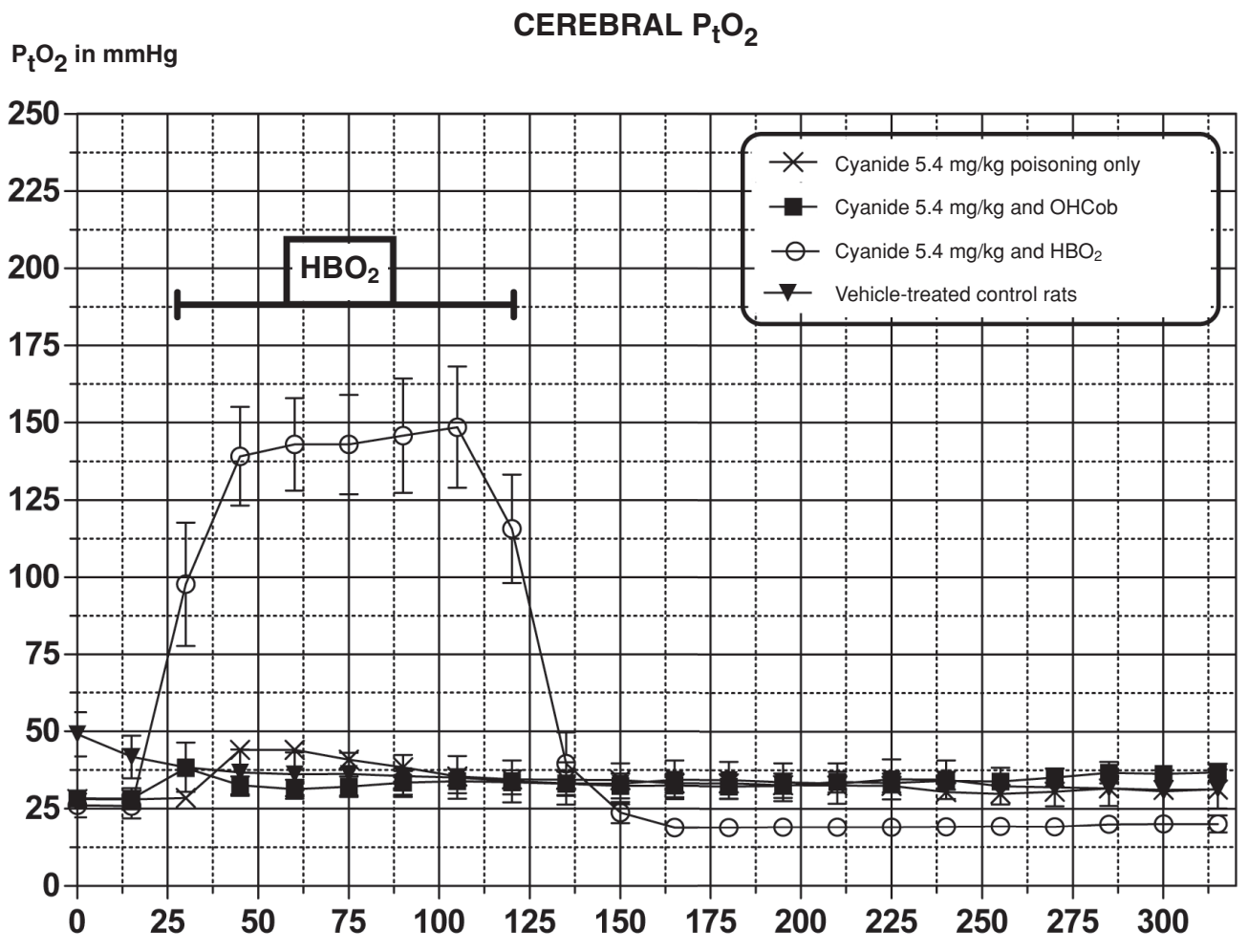

FIGURE 1 – P_tO_2 measurements in rat brain during either: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO_2 (284 kPa for 90 minutes). Horizontal bar and square box showing time of 90 minutes' HBO_2 treatment. Mean values are given ± SEM.

HBO_2: CN-intoxicated rats with respiratory distress and cyanosis which were subsequently given HBO_2 treatment showed a fast improvement in respiration and disappearance of cyanosis. Once HBO_2 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 HBO_2 treatments, MAP would rise from 100-135 mmHg to 140-150 mmHg, as the most frequent interval and remained at this level. After HBO_2 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 HBO_2, a major rise in P_tO_2 of about 70-100 mmHg to an average of 140 mm Hg was observed. The tissue hyperoxia remained stable until the HBO_2 therapy was ceased and from which point the P_tO_2 returned to pre-HBO_2 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 ± SD	LACTATE *Mean interstitial cerebral lactate concentration in mmol/l ** *values given ± SD*		GLUCOSE *Mean interstitial cerebral glucose concentration in mmol/l ** *values given ± SD*	
Treatment groups *(N=number of rats)* **Time**	**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 ± 0.05 [1]	0.44 ± 0.05	0.25 ± 0.07	0.31 ± 0.03 [7]
KCN (*N* =10)	0.66 ± 0.13 [2]	0.27 ± 0.10 [3]	0.31 ± 0.12	0.25 ± 0.11
KCN + OHCob (*N* =9)	0.42 ± 0.12	0.22 ± 0.03 [4]	0.13 ± 0.05 [5]	0.07 ± 0.02 [8]
KCN + HBO_2 (*N* =11)	0.47 ± 0.12	0.42 ± 0.05	0.16 ± 0.03 [6]	0.17 ± 0.04 [9]

[1] Controls vs KCN $P<0.001$. [2] KCN vs OHCob and HBO_2; $P<0.001$ and $P<0.01$ respectively. [3] KCN vs Control and HBO_2; $P<0.001$ and $P<0.001$ respectively. [4] OHCob vs Control and HBO_2; $P<0.001$ and $P<0.001$ respectively. [5] OHCob vs Controls and KCN; $P<0.01$and $P<0.001$ respectively. [6] HBO_2 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 HBO_2; v<0.001for all comparisons. [9] HBO_2 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 HBO_2 after CN infusion: The animals given CN i.a. at *t*=15 minutes and then treated with HBO_2 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-HBO_2 observation period increased slowly *(Figure 3)*. During and after HBO_2 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 HBO_2

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 HBO_2 were not different from each other ($P>0.05$).

FIGURE 4

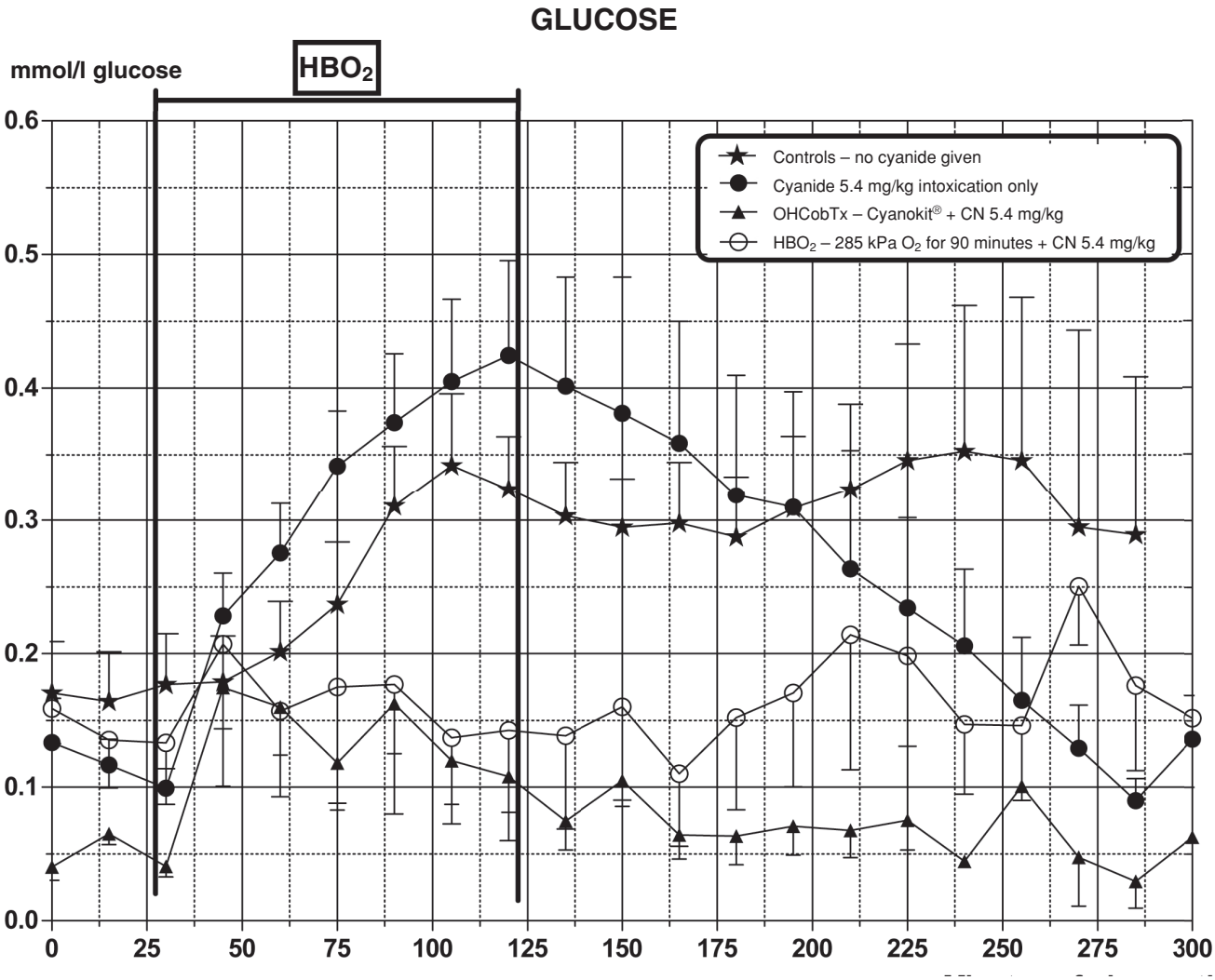

FIGURE 4 – Changes in interstitial cerebral glucose concentrations during either: 1) vehicle-treated control rats; 2) KCN-poisoned rats; 3) KCN-poisoned rats receiving hydroxycobalamin (25mg); and 4) KCN-poisoned rats treated with HBO_2 (284 kPa for 90 minutes). Horizontal bar and square box showing time of 90 minutes HBO_2 treatment. Mean values are given ± SEM.

However, in rats treated with either OHCob or HBO_2 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 HBO_2-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 HBO_2-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 HBO_2-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 HBO_2-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 HBO_2 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 HBO_2-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 HBO_2-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 HBO_2-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 HBO_2-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 HBO_2 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_tO_2 in certain regions of the rat brain [28,29]. This HBO_2-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_tO_2 increased significantly during HBO_2 treatments at 285 kPa in these experiments *(Figure 1)* suggests that regional cerebral blood flow was not affected to a degree causing reduced P_tO_2.

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 HBO_2 [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 metabolization 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 a,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 HBO_2 is uncertain. Also the synergistic effect of HBO_2 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, HBO_2, but not normobaric oxygen, was shown to improve mitochondrial oxidative processes during CN poisoning [51]. Irrespective of the contradictory results, HBO_2 has been shown to improve survival and improve tissue oxygenation in the clinical as well as in the experimental settings [52] and HBO_2 is recommended especially when supportive measures and other CN antidotes fail [50,53.54]. Furthermore, we recently demonstrated that HBO_2 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 a, 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, HBO_2, 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, HBO_2 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 HBO_2 therapy [12].

In the present results we observed that HBO_2 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 HBO_2 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 HBO_2 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 HBO_2 *(Figure 4)*. This seemingly protracted increase in glucose concentration after HBO_2, in comparison to OHCob, may reflect a continued CN intoxication once HBO_2 treatment has seized, since HBO_2 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 HBO_2 treatment administered immediately after CN intoxication ameliorates the interstitial surge seen in cerebral lactate and glucose concentrations during treatment. The effect of HBO_2 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 HBO_2 treatment increased cerebral P_tO_2 and reduced respiratory distress and cyanosis, OHCob did not have this beneficial effect. Since HBO_2 is not a CN scavenger, the effect of HBO_2 may be mediated through $^{\bullet}NO$ 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 HBO_2 treatment has subsided. Accordingly, further studies on the combined effect of HBO_2 and OHCob treatment are warranted.

Acknowledgements

The technical assistance of hyperbaric technician and dive supervisor Mr. Michael Bering Sifakis is greatly appreciated. This study was supported by grants from Tryg Foundation and The A.P. Møller Foundation for the Advancement of Medical Science.

■

REFERENCES

1. Gracia R, Shepherd G. Cyanide poisoning and its treatment. Pharmacotherapy 2004 October;24(10):1358-65.

2. Baud FJ. Cyanide: critical issues in diagnosis and treatment. Hum Exp Toxicol 2007 March;26(3):191-201.

3. Baud FJ, Borron SW, Megarbane B, Trout H, Lapostolle F, Vicaut E, Debray M, Bismuth C. Value of lactic acidosis in the assessment of the severity of acute cyanide poisoning. Crit Care Med 2002 September;30(9):2044-50.

4. Rachinger J, Fellner FA, Stieglbauer K, Trenkler J. MR changes after acute cyanide intoxication. AJNR Am J Neuroradiol 2002 September;23(8):1398-401.

5. Megarbane B, Delahaye A, Goldgran-Toledano D, Baud FJ. Antidotal treatment of cyanide poisoning. J Chin Med Assoc 2003 April;66(4):193-203.

6. Stubauer G, Giuffre A, Brunori M, Sarti P. Cytochrome c oxidase does not catalyze the anaerobic reduction of NO. Biochem Biophys Res Commun 1998 April 17;245(2):459-65.

7. Pearce LL, Bominaar EL, Hill BC, Peterson J. Reversal of cyanide inhibition of cytochrome c oxidase by the auxiliary substrate nitric oxide: an endogenous antidote to cyanide poisoning? J Biol Chem 2003 December 26;278(52):52139-45.

8. Allen BW, Demchenko IT, Piantadosi CA. Two faces of nitric oxide: implications for cellular mechanisms of oxygen toxicity. J Appl Physiol 2009 February;106(2):662-7.

9. Ohgami Y, Chung E, Shirachi DY, Quock RM. The effect of hyperbaric oxygen on regional brain and spinal cord levels of nitric oxide metabolites in rat. Brain Res Bull 2008 March 28;75(5):668-73.

10. Thom SR, Bhopale V, Fisher D, Manevich Y, Huang PL, Buerk DG. Stimulation of nitric oxide synthase in cerebral cortex due to elevated partial pressures of oxygen: an oxidative stress response. J Neurobiol 2002 May;51(2):85-100.

11. Thom SR, Fisher D, Zhang J, Bhopale VM, Ohnishi ST, Kotake Y, Ohnishi T, Buerk DG. Stimulation of perivascular nitric oxide synthesis by oxygen. Am J Physiol Heart Circ Physiol 2003 April;284(4):H1230-H1239.

12. Lawson-Smith P. Effect of acute and delayed hyperbaric oxygen therapy on cyanide whole blood levels during acute cyanide intoxication. J Undersea Hyperb Med 2011;38(1):49-58.

13. Forsyth JC, Mueller PD, Becker CE, Osterloh J, Benowitz NL, Rumack BH, Hall AH. Hydroxocobalamin as a cyanide antidote: safety, efficacy and pharmacokinetics in heavily smoking normal volunteers. J Toxicol Clin Toxicol 1993;31(2):277-94.

14. Way JL. Cyanide intoxication and its mechanism of antagonism. Annu Rev Pharmacol Toxicol 1984;24:451-81.

15. Van den Berg MP, Merkus P, Romeijn SG, Verhoef JC, Merkus FW. Hydroxocobalamin uptake into the cerebrospinal fluid after nasal and intravenous delivery in rats and humans. J Drug Target 2003 July;11(6):325-31.

16. Houeto P, Borron SW, Sandouk P, Imbert M, Levillain P, Baud FJ. Pharmacokinetics of hydroxocobalamin in smoke inhalation victims. J Toxicol Clin Toxicol 1996;34(4):397-404.

17. Alm A, Tornquist P. Lactate transport through the blood-retinal and the blood-brain barrier in rats. Ophthalmic Res 1985;17(3):181-4.

18. Gjedde A, Andersson J, Eklof B. Brain uptake of lactate, antipyrine, water and ethanol. Acta Physiol Scand 1975 February;93(2):145-9.

19. Borowitz JL, Rathinavelu A, Kanthasamy A, Wilsbacher J, Isom GE. Accumulation of labeled cyanide in neuronal tissue. Toxicol Appl Pharmacol 1994 November;129(1):80-5.

20. Hyldegaard O, Kerem D, Melamed Y. Effect of combined recompression and air, oxygen, or heliox breathing on air bubbles in rat tissues. J Appl Physiol 2001 May;90(5):1639-47.

21. Ibrahim MZ, Briscoe PB, Jr., Bayliss OB, Dams CW. The relationshipbetween enzyme activityand neuroglia in the prodromal and demyelinating stages of cyanide encephalopathy in the rat. J Neurol Neurosurg Psychiatry 1963 December;26: 479-86.

22. Lawson-Smith P, Jansen EC, Hilsted L, Johnsen AH, Hyldegaard O. Treatment of Cyanide Poisoning with HBO_2. Undersea Hyperbaric Med 2010 October 1;37(5).

23. SPSS. Statistical Package of the Social Science. Chicago, IL: SPSS; 1998.

24. Altman DG. Practical Statistics for Medical Research. London, UK: Chapman and Hall; 1991.

25. Alarie Y. Toxicity of fire smoke. Crit Rev Toxicol 2002 July;32(4):259-89.

26. Jones J, McMullen MJ, Dougherty J. Toxic smoke inhalation: cyanide poisoning in fire victims. Am J Emerg Med 1987 July;5(4):317-21.

27. Devlin DJ, Smith RP, Thron CD. Cyanide metabolism in the isolated, perfused, bloodless hindlimbs or liver of the rat. Toxicol Appl Pharmacol 1989 April;98(2):338-49.

28. Bergo GW, Tyssebotn I. Cerebral blood flow distribution during exposure to 5 bar oxygen in awake rats. Undersea Biomed Res 1992 September;19(5):339-54.

29. Bergo GW, Tyssebotn I. Regional cerebral blood flow during exposure to 1, 3 and 5 bar oxygen. Undersea and Hyperbaric Medical Society, Inc 15[No 1 supplement]. 1989. Ref Type: Abstract

30. Bequet F, Peres M, Gomez-Merino D, Berthelot M, Satabin P, Pierard C, Guezennec CY. Simultaneous NMR microdialysis study of brain glucose metabolism in relation to fasting or exercise in the rat. J Appl Physiol 2000 June;88(6):1949-54.

31. Fray AE, Forsyth RJ, Boutelle MG, Fillenz M. The mechanisms controlling physiologically stimulated changes in rat brain glucose and lactate: a microdialysis study. J Physiol 1996 October 1;496 (Pt 1):49-57.

32. Johansen O, Vaaler S, Jorde R, Reikeras O. Increased plasma glucose levels after Hypnorm anaesthesia, but not after pentobarbital anaesthesia in rats. Lab Anim 1994 July;28(3): 244-8.

33. Al-Waili NS, Butler GJ, Beale J, Abdullah MS, Finkelstein M, Merrow M, Rivera R, Petrillo R, Carrey Z, Lee B, Allen M. Influences of hyperbaric oxygen on blood pressure, heart rate and blood glucose levels in patients with diabetes mellitus and hypertension. Arch Med Res 2006 November;37(8):991-7.

34. Gu N, Nagatomo F, Fujino H, Takeda I, Tsuda K, Ishihara A. Hyperbaric oxygen exposure improves blood glucose level and muscle oxidative capacity in rats with type 2 diabetes. Diabetes Technol Ther 2010 February;12(2):125-33.

35. Clausen T, Zauner A, Levasseur JE, Rice AC, Bullock R. Induced mitochondrial failure in the feline brain: implications for understanding acute post-traumatic metabolic events. Brain Res 2001 July 20;908(1):35-48.

36. Akyildiz BN, Kurtoglu S, Kondolot M, Tunc A. Cyanide poisoning caused by ingestion of apricot seeds. Ann Trop Paediatr 2010;30(1):39-43.

37. Yessoufou A, Ategbo JM, Girard A, Prost J, Dramane KL, Moutairou K, Hichami A, Khan NA. Cassava-enriched diet is not diabetogenic rather it aggravates diabetes in rats. Fundam Clin Pharmacol 2006 December;20(6):579-86.

38. Klimmek R, Roddewig C, Fladerer H, Weger N. Cerebral blood flow, circulation, and blood homeostasis of dogs during slow cyanide poisoning and after treatment with 4-dimethylaminophenol. Arch Toxicol 1982 May;50(1):65-76.

39. Klimmek R, Roddewig C, Fladerer H, Krettek C, Weger N. Effects of 4-dimethylaminophenol, Co2EDTA, or NaNO2 on cerebral blood flow and sinus blood homeostasis of dogs in connection with acute cyanide poisoning. Toxicology 1983 February;26(2):143-54.

40. Frykholm P, Hillered L, Langstrom B, Persson L, Valtysson J, Enblad P. Relationship between cerebral blood flow and oxygen metabolism, and extracellular glucose and lactate concentrations during middle cerebral artery occlusion and reperfusion: a microdialysis and positron emission tomography study in nonhuman primates. J Neurosurg 2005 June;102(6):1076-84.

41. Pettersen JC, Cohen SD. The effects of cyanide on brain mitochondrial cytochrome oxidase and respiratory activities. J Appl Toxicol 1993 January;13(1):9-14.

42. Hall AH, Rumack BH. Hydroxycobalamin/sodium thiosulfate as a cyanide antidote. J Emerg Med 1987;5(2): 115-21.

43. Bebarta VS, Tanen DA, Lairet J, Dixon PS, Valtier S, Bush A. Hydroxocobalamin and sodium thiosulfate versus sodium nitrite and sodium thiosulfate in the treatment of acute cyanide toxicity in a swine (Sus scrofa) model. Ann Emerg Med 2010 April;55(4):345-51.

44. Gerth K, Ehring T, Braendle M, Schelling P. Nitric oxide scavenging by hydroxocobalamin may account for its hemodynamic profile. Clin Toxicol (Phila) 2006;44 Suppl 1:29-36.

45. Gourlain H, Caliez C, Laforge M, Buneaux F, Levillain P. Study of the mechanisms involved in hydroxocobalamin interference with determination of some biochemical parameters. Ann Biol Clin (Paris) 1994;52(2):121-4.

46. Coburn RF, Grubb B, Aronson RD. Effect of cyanide on oxygen tension-dependent mechanical tension in rabbit aorta. Circ Res 1979 March;44(3):368-78.

47. Piantadosi CA, Sylvia AL, Jobsis FF. Cyanide-induced cytochrome a,a3 oxidation-reduction responses in rat brain in vivo. J Clin Invest 1983 October;72(4):1224-33.

48. Yonetani T, RAY GS. Studies on cytochrome oxidase. VI. Kinetics of the aerobic oxidation of ferrocytochrome c by cytochrome oxidase. J Biol Chem 1965 August;240:3392-8.

49. Sorbo B. On the mechanism of rhodanese inhibition by sulfite and cyanide. Acta Chem Scand 1962;2455-6.

50. Way JL, End E, Sheehy MH, De MP, Feitknecht UF, Bachand R, Gibbon SL, Burrows GE. Effect of oxygen on cyanide intoxication. IV. Hyperbaric oxygen. Toxicol Appl Pharmacol 1972 July;22(3):415-21.

51. Takano T, Miyazaki Y, Nashimoto I, Kobayashi K. Effect of hyperbaric oxygen on cyanide intoxication: in situ changes in intracellular oxidation reduction. Undersea Biomed Res 1980 September;7(3):191-7.

52. Davis FM, Ewer T. Acute cyanide poisoning: case report of the use of hyperbaric oxygen. 3, 103-106. 1988. J Hyper Med. Ref Type: Generic

53. Hall AH, Rumack BH. Clinical toxicology of cyanide. Ann Emerg Med 1986 September;15(9):1067-74.

54. Thom SR, Keim LW. Carbon monoxide poisoning: a review epidemiology, pathophysiology, clinical findings, and treatment options including hyperbaric oxygen therapy. J Toxicol Clin Toxicol 1989;27(3):141-56.

55. Ikegaya H, Iwase H, Hatanaka K, Sakurada K, Yoshida K, Takatori T. Diagnosis of cyanide intoxication by measurement of cytochrome c oxidase activity. Toxicol Lett 2001 February 28;119(2):117-23.

56. Brown SD, Piantadosi CA. Recovery of energy metabolism in rat brain after carbon monoxide hypoxia. J Clin Invest 1992 February;89(2):666-72.

57. Ducasse JL, Celsis P, Marc-Vergnes JP. Non-comatose patients with acute carbon monoxide poisoning: hyperbaric or normobaric oxygenation? Undersea Hyperb Med 1995 March;22(1):9-15.

58. Thom SR. Antagonism of carbon monoxide-mediated brain lipid peroxidation by hyperbaric oxygen. Toxicol Appl Pharmacol 1990 September 1;105(2):340-4.

59. Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG. Stem cell mobilization by hyperbaric oxygen. Am J Physiol Heart Circ Physiol 2006 April;290(4): H1378-H1386.

60. Weaver LK, Hopkins RO, Chan KJ, Churchill S, Elliott CG, Clemmer TP, Orme JF, Jr., Thomas FO, Morris AH. Hyperbaric oxygen for acute carbon monoxide poisoning. N Engl J Med 2002 October 3;347(14):1057-67.

61. Averill BA. Dissimilatory nitrite and nitric oxide reductases. Chem Rev 1996 November 7;96(7):2951-64.

62. Pearce LL, Lopez ME, Martinez-Bosch S, Peterson J. Antagonism of nitric oxide toward the inhibition of cytochrome c oxidase by carbon monoxide and cyanide. Chem Res Toxicol 2008 November;21(11):2073-81.

63. Xu X, Wang Z, Li Q, Xiao X, Lian Q, Xu W, Sun X, Tao H, Li R. Endothelial nitric oxide synthase expression is progressively increased in primary cerebral microvascular endothelial cells during hyperbaric oxygen exposure. Oxid Med Cell Longev 2009 January;2(1):7-13.

64. Schurr A, West CA, Rigor BM. Lactate-supported synaptic function in the rat hippocampal slice preparation. Science 1988 June 3;240(4857):1326-8.

65. Schurr A. Lactate: the ultimate cerebral oxidative energy substrate? J Cereb Blood Flow Metab 2006 January;26(1): 142-52.

66. Berthet C, Lei H, Thevenet J, Gruetter R, Magistretti PJ, Hirt L. Neuroprotective role of lactate after cerebral ischemia. J Cereb Blood Flow Metab 2009 November;29(11):1780-9.

67. Ichai C, Armando G, Orban JC, Berthier F, Rami L, Samat-Long C, Grimaud D, Leverve X. Sodium lactate versus mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. Intensive Care Med 2009 March;35(3):471-9.

68. DeSalles AA, Kontos HA, Becker DP, Yang MS, Ward JD, Moulton R, Gruemer HD, Lutz H, Maset AL, Jenkins L. Prognostic significance of ventricular CSF lactic acidosis in severe head injury. J Neurosurg 1986 November;65(5):615-24.

❑