Intensive Care Med (2012) 38:1877–1885
DOI 10.1007/s00134-012-2714-x

EXPERIMENTAL

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Effects of exogenous hydrogen sulfide on brain metabolism and early neurological function in rabbits after cardiac arrest

Received: 3 April 2012
Accepted: 9 September 2012
Published online: 26 September 2012

Electronic supplementary material
The online version of this article (doi:10.1007/s00134-012-2714-x) contains supplementary material, which is available to authorized users.

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Abstract *Purpose:* Some of the neuroprotective effects of hydrogen sulfide (H_2S) have been attributed to systemic hypometabolism and hypothermia. However, systemic metabolism may vary more dramatically than brain metabolism after cardiac arrest (CA). The authors investigated the effects of inhaled exogenous hydrogen sulfide on brain metabolism and neurological function in rabbits after CA and resuscitation. *Methods:* Anesthetized rabbits were randomized into a sham group, a sham/H_2S group, a CA group, and a CA/H_2S group. Exogenous 80 ppm H_2S was administered to the sham/H_2S group and the CA/H_2S group which suffered 3 min of untreated CA by asphyxia and resuscitation. Effects on brain metabolism (cerebral extraction of oxygen (CEO_2), arteriojugular venous difference of glucose [AJVD(glu)] and lactate clearance), S100B, viable neuron counts, neurological dysfunction score, and survival rate were evaluated. *Results:* CEO_2, AJVD(glu), and lactate increased significantly after CA. Inhalation of 80 ppm H_2S significantly increased CEO_2 (25.04 ± 7.11 vs. 16.72 ± 6.12 %) and decreased AJVD(glu) (0.77 ± 0.29 vs. 1.18 ± 0.38 mmol/L) and lactate (5.11 ± 0.43 vs. 6.01 ± 0.64 mmol/L) at 30 min after resuscitation when compared with the CA group (all $P < 0.05$). In addition, neurologic deficit scores, viable neuron counts, and survival rate were significantly better whereas S100B was decreased after H_2S inhalation. *Conclusions:* The present study reveals that inhalation of 80 ppm H_2S reduced neurohistopathological damage and improves early neurological function after CA and resuscitation in rabbits. The increased CEO_2 and decreased AJVD(glu) and enhanced lactate clearance may be involved in the protective effects.

Keywords Hydrogen sulfide · Brain metabolism · Neurologic manifestations · Heart arrest · Resuscitation

Introduction

Over the last decade, hydrogen sulfide (H_2S) has been recognized as a gasotransmitter, playing an important role in brain function [1–3]. Endogenous H_2S can be produced from cysteine by cystathionine β-synthase (CBS) in the central nervous system and cystathionine γ-lyase (CSE) in the vessels and other tissues. Recently, higher levels of endogenous H_2S have been detected in the brain than in other organs. This suggests that H_2S may have a neuromodulatory role [3].

Some of the neuroprotective effects of H_2S can be attributed to hypometabolism and hypothermia. For

example, Blackstone et al. [1] observed that inhalation of H_2S decreased the brain metabolic rate in mice by more than 50 % within 5 min. The metabolic rate is also associated with reductions in body temperature. However, the applicability of murine models of metabolism to larger animals has been questioned [4–6]. Furthermore, all the aforementioned studies focused on either whole-body metabolism or core temperature. However, changes in systemic metabolism, including oxygen extraction after cardiac arrest (CA), may vary more dramatically than brain metabolism [7]. To predict neurological outcomes, brain metabolism, especially cerebral extraction of oxygen (CEO_2), must be taken into account to a greater extent than systemic metabolism [8, 9]. However, studies of brain metabolism and the effects of H_2S on brain metabolism are rare. The effects of H_2S on neurological function after CA and resuscitation remain poorly understood.

The aim of this study was to investigate the effects of inhaled exogenous H_2S on brain metabolism and neurological function in rabbits after CA and resuscitation.

Materials and methods

For detailed materials and methods, see the electronic supplementary material (ESM).

Animal surgical procedures

This study was approved by the Institutional Animal Care and Use Committee of Harbin Medical University in Harbin, Heilongjiang, China, and followed national guidelines for the treatment of animals. Adult male New Zealand white rabbits, weighing 2.5–3.0 kg, were used in this study. Food and water were available ad libitum until the morning of the experiment.

Anesthesia was performed with 1–2 % halothane and 30 % oxygen. Ventilation was controlled with a small animal ventilator (Kent Scientific, Litchfield, CT, USA) to maintain arterial pH at 7.35–7.45, $PaCO_2$ at 35–45 mmHg, and PaO_2 over 90 mmHg. All animals were mechanically ventilated except during the asphyxial CA procedures. Rectal temperatures were monitored continuously with thermal probes (BIOPAC Systems Inc., Santa Barbara, CA, USA) and the ambient temperature of 25 °C was maintained throughout the experiment. A standard lead II ECG was recorded continuously using subdermal needle-type electrodes placed in the limbs. All of the physiology data were monitored and recorded using the BIOPAC MP150 physiometer (BIOPAC Systems Inc., Santa Barbara, CA, USA).

Two saline-filled 18-gauge polyethylene catheters were inserted into the carotid artery for blood pressure measurement and into the left femoral vein for infusion and drug administration. Another 18-gauge catheter was placed in the right internal jugular vein and advanced in the retrograde direction until increased resistance was met at the base of the skull. The catheter was then withdrawn a few millimeters and secured, after which the blood flowed readily. The position of the catheter tip was at the right internal jugular bulb and confirmed by autopsy in animals that failed resuscitative efforts.

After preparation and subsequent stabilization, the animals were randomly assigned to four groups: sham group, sham/H_2S group, CA group, and CA/H_2S group (details in ESM).

Cardiac arrest and resuscitation

Cardiac arrest was induced in CA and CA/H_2S groups by asphyxia as previously described [10, 11]. Asphyxia was induced by administering an additional dose of vecuronium and clamping the endotracheal tube. The cardiac arrest period was considered to begin when MAP decreased to less than 10 mmHg and left untreated for 3 min. Total asphyxia time was approximately 8 min. A detailed description of this model including the exact resuscitative procedures is provided in the ESM.

Measurement of brain temperature

Two digital electronic thermometers with thermocouple probes were inserted for continuous monitoring of the cortex and hippocampus temperature (details in ESM).

Determination of S100B level

Blood samples were draw from the internal jugular venous bulb and centrifuged for measuring the concentrations of S100B with a sandwich ELISA kit (details in ESM).

Neurological assessment and survival rate

All animals were evaluated before the experiment to ensure normal neurological function. Neurological dysfunction was evaluated daily in surviving animals with a clinical score previously validated in rabbits (0–10 % normal, 100 % brain death) [12]. Neurological dysfunction score (NDS) and the survival rates were assessed by the same investigator who was unaware of the group assignment during the 7 days after return of spontaneous circulation (ROSC).

Neuronal counts

On the 7th day after ROSC, the rabbits were deeply anesthetized with halothane. Hippocampi were removed and sectioned to 3 μm for hematoxylin and eosin staining. The numbers of viable neurons were counted in the CA1 region per high-power field (×400) (details in ESM).

Concentration of hydrogen sulfide

The concentrations of H_2S in the plasma at baseline (t0), 30 min after ROSC (t1), and 60 min after ROSC (t2) were measured according to the method described previously (details in ESM) [13].

Measurement of brain metabolism

Cerebral extraction of oxygen (CEO_2), arterio-jugular venous differences of glucose [AJVD(glu)], and lactate clearance were measured at t0, t1, and t2. A detailed description of brain metabolism is provided in the ESM.

Statistical analysis

Data are presented as mean ± SD. Intergroup differences in temperature, H_2S concentration, CEO_2, AJVD(glu), lactate, and S100B were assessed by two-way repeated-measures analysis of variance (ANOVA) with Bonferroni post hoc test. Differences in lactate clearance and neuron numbers were analyzed by one-way ANOVA with Bonferroni correction for post hoc comparison between multiple experimental groups. NDSs were compared with those of the corresponding CA group by use of a Mann–Whitney nonparametric test. Kaplan–Meier survival curves were compared using log-rank test. Significance was considered at the level of $P < 0.05$. Statistical analysis was performed using SPSS® software (SPSS Inc., Chicago, IL, USA).

Results

Forty-five rabbits were included in the present study. Twelve of 15 rabbits were successfully resuscitated in the CA and CA/H_2S groups. The characteristics of CA and resuscitation showed no statistical differences between the CA and CA/H_2S groups (ESM Table 1). There were no significant differences in physiological variables among all groups at the baseline period (ESM Table 2).

Rectal and cerebral temperature

Although the temperature was slightly lower in the H_2S treated animals, no statistical differences were observed in rectal, cortex, and hippocampus temperatures between CA and CA/H_2S groups during the 60 min after ROSC. There were also no significant differences in rectal, cortex, and hippocampus temperature between sham and sham/H_2S groups (Fig. 1).

S100B

There was no significant difference in S100B between sham and sham/H_2S groups. The level of serum S100B was significantly higher in the CA group than in the sham group at t1 (9.69 ± 1.50 vs. 7.00 ± 1.06 ng/ml, $P < 0.05$) and t2 (14.16 ± 1.99 vs. 6.99 ± 0.97 ng/ml, $P < 0.05$). However, this increase was significantly less pronounced in the CA/H_2S group at t1 (8.63 ± 1.19 ng/ml) and t2 (10.85 ± 1.44 ng/ml) than in the CA group (both $P < 0.05$) (Fig. 2).

NDS and overall survival rate after ROSC

A significant difference in survival rate was shown between the CA/H_2S group and CA group ($P < 0.05$) (Fig. 3a). At the end of the follow-up, five rabbits [42 % (5 of 12)] in the CA group and 10 rabbits [83 % (10 of 12)] in the CA/H_2S group survived until day 7. It is noteworthy that the deaths occurred primarily between days 2 and 4. This is a time when death due to CA causes is uncommon and neurological causes predominate.

As shown in Fig. 3b, at 24 h after ROSC, the NDS was significantly attenuated in the CA/H_2S group compared with the CA group ($P < 0.05$) (Fig. 3b).

Neuronal counts

On the 7th day after CA and resuscitation, the neuronal density of the CA group was much lower than that of the sham group (Fig. 4a). The pyramidal neurons appeared swollen and arranged in irregular patterns. Nuclear pyknosis, karyorrhexis, and vacuolization were also observed. However, neuronal density and cell morphology were well preserved in the CA/H_2S group. For comparison, the numbers of viable neurons per high-power field (×400) in the CA and CA/H_2S groups were significantly lower than that of the sham group (both $P < 0.05$). The hippocampal CA1 counts were 24 ± 6 in the CA/H_2S group, which was significantly more than in the CA group (10 ± 3, $P < 0.05$) but still less than in the sham group (39 ± 6,

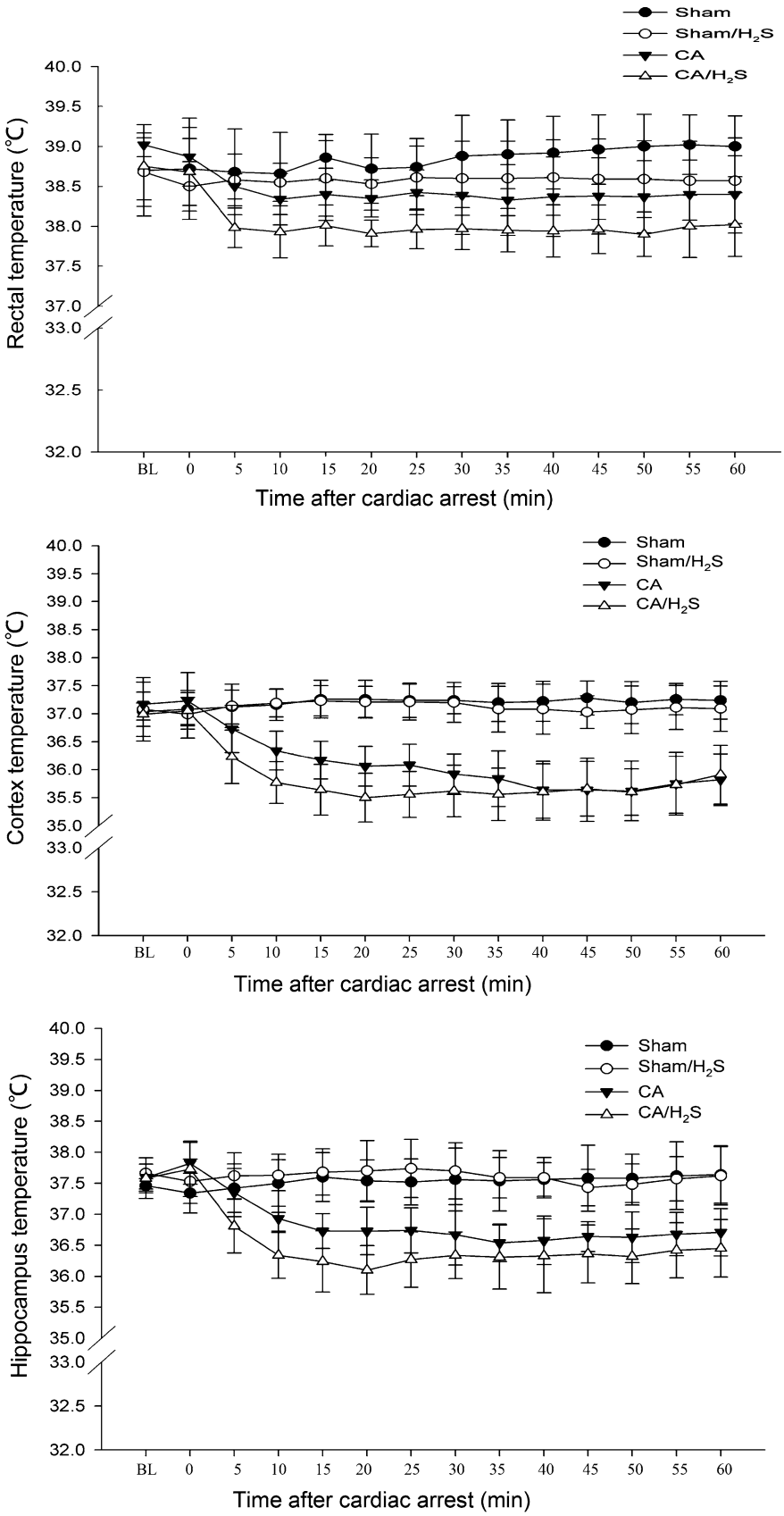

Fig. 1 Rectal, cortex, and hippocampus temperatures in the different experimental groups. *BL* baseline

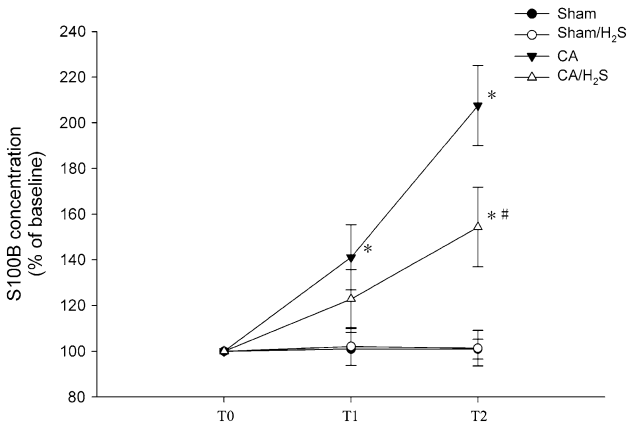

Fig. 2 Concentration of S100B as percentage of baseline values at t0, t1, and t2 for each group. *T0*, *T1*, and *T2* represent baseline, 30 and 60 min after restoration of spontaneous circulation. $^{*}P < 0.05$ versus sham group. $^{\#}P < 0.05$ versus CA group

$P < 0.05$). No significant differences in CA1 neuron counts were observed between the sham and sham/H_2S groups (Fig. 4b).

H_2S concentration

As can be seen in Fig. 5, the concentration of H_2S was significantly increased after inhalation of exogenous H_2S in the sham/H_2S group at t1 and t2 (3.26 ± 0.47 and 3.74 ± 0.29 μmol/L, respectively) compared with the sham group (2.29 ± 0.07, 2.38 ± 0.14 μmol/L, respectively, both $P < 0.05$). These changes were also observed between the CA and CA/H_2S groups at t1 (2.63 ± 0.33 vs. 6.43 ± 0.51 μmol/L) and t2 (2.92 ± 0.41 vs. 8.16 ± 0.38 μmol/L) (both $P < 0.05$). We also observed higher concentrations of H_2S in the CA group relative to the sham group at t1 and t2. This difference was significant at t2 ($P < 0.05$). The levels of H_2S with H_2S therapy were significantly higher in animals undergoing CA compared to shams ($P < 0.05$ for both t1 and t2).

Brain metabolic measures

CEO_2

As presented in Fig. 6, the levels of CEO_2 at t1 were 16.72 ± 6.12 % in the CA group and 25.04 ± 7.11 % in the CA/H_2S group. These were both significantly higher than those of the sham group (8.46 ± 4.73 %, both $P < 0.05$). This tendency was still observable at t2 (19.69 ± 6.02, 22.36 ± 7.70, and 9.31 ± 3.29 %, respectively, $P < 0.05$). The CA/H_2S group had a higher level of CEO_2 than the CA group at t1 ($P < 0.05$) and t2. No significant differences were observed between the sham and sham/H_2S groups throughout the 60 min of the experiment.

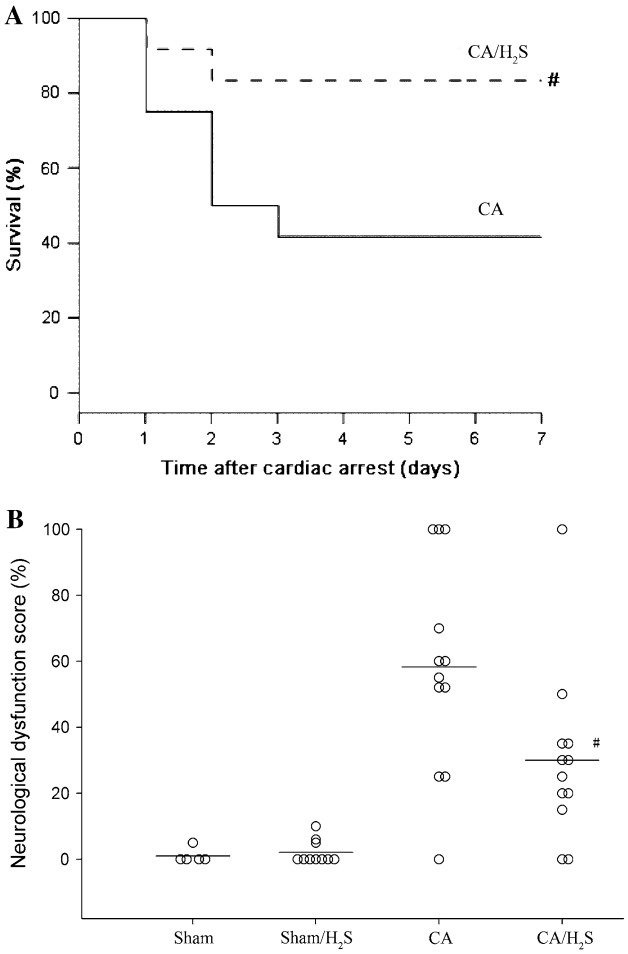

Fig. 3 **a** Kaplan–Meier survival curves in the different experimental groups submitted to cardiac arrest and resuscitation. **b** Neurological dysfunction scores at day 1 after resuscitation in the different experimental groups. *Open circles* represent individual scores; *thick line* the median value of the corresponding group. $^{\#}P < 0.05$ versus CA group

Arterio-jugular venous differences in glucose concentration

The values of AJVD(glu) were significantly higher in the CA group (1.18 ± 0.38 mmol/L, $P < 0.05$) but only slightly higher in the CA/H_2S group (0.77 ± 0.29 mmol/L) than sham values (0.44 ± 0.14 mmol/L) at t1. There was a significant decrease in AJVD(glu) in the CA/H_2S group compared with the CA group at t1 ($P < 0.05$). The increase in AJVD(glu) in the CA group returned to normal values at t2 (Fig. 6). No significant differences in AJVD(glu) were observed between the sham and sham/H_2S groups at any time.

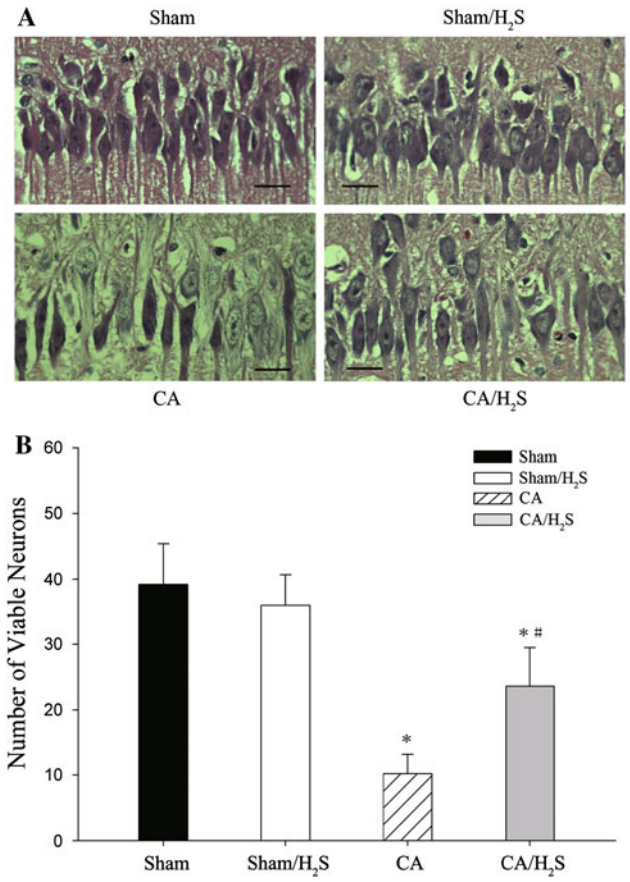

Fig. 4 **a** Representative histological images of the hippocampus CA1 region from sham, sham/H_2S, CA, and CA/H_2S groups at 7 days after restoration of spontaneous circulation. All images were captured at ×400 magnification. *Scale bar* indicates 100 μm. **b** Number of viable neurons in the CA1 region belonging to different groups. $^{*}P < 0.05$ versus sham group. $^{\#}P < 0.05$ versus CA group

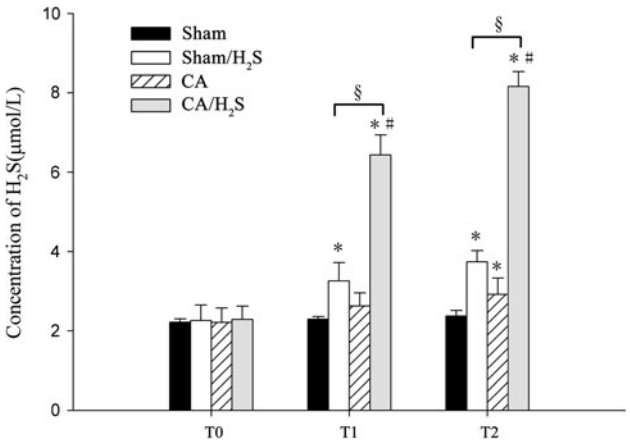

Fig. 5 Concentration of plasma H_2S for each group. *T0*, *T1*, and *T2* represent baseline, 30 and 60 min after restoration of spontaneous circulation. $^{*}P < 0.05$ versus sham group. $^{\#}P < 0.05$ versus CA group, $^{\S}P < 0.05$ versus sham/H_2S group

Lactate and lactate clearance

A statistically significantly higher lactate level was observed in the CA (6.01 ± 0.64 mmol/L) and CA/H_2S group (5.11 ± 0.43 mmol/L) at t1 than in the sham group (3.50 ± 0.33 mmol/L, both $P < 0.05$). Differences in lactate levels between the CA and CA/H_2S groups reached statistical significance at t1 ($P < 0.05$) and widened by t2 (6.11 ± 0.77 vs. 3.83 ± 0.56 mmol/L, $P < 0.05$) (Fig. 6).

After ROSC, lactate clearance of Lactate (t0–t1) and Lactate (t0–t2) were negative, indicating an increase in lactate level. However, in the CA/H_2S group, Lactate (t1–t2) was positive, indicating lactate clearance after ROSC. As shown in Fig. 6, the values of Lactate (t0–t1) (74.22 ± 12.59 %) and Lactate (t0–t2) (77.04 ± 28.20 %) were significantly higher in the CA group than in the sham group (0.48 ± 5.76, 3.49 ± 6.93 %, both $P < 0.05$). However, Lactate (t0–t1) (45.98 ± 13.43 %) and Lactate (t0–t2) (9.72 ± 19.67 %) were significantly lower in CA/H_2S group than in the CA group (both $P < 0.05$).

Discussion

The current study investigated the effects of exogenous H_2S on cerebral metabolism and neurological function following CA and resuscitation. The key findings were that H_2S reduced neurohistopathological damage and improved early neurological function after CA and resuscitation. Increased CEO_2, decreased AJVD(glu), and enhanced lactate clearance were observed at the same time.

In this study, we observed that inhaling exogenous 80 ppm H_2S gas significantly increased the concentration of H_2S in plasma. Interestingly, we observed an increase of H_2S after ROSC even without inhalation of exogenous H_2S in the CA group. Several researchers have described significant changes of H_2S in plasma levels in various disease states [14–16]. A recent study with cerebellar slices and intact mouse brains identified that during hypoxia inhibition of heme oxygenase 2 mediated CO production, with a corresponding release of the tonic inhibition of CBS, and allowing CBS to generate H_2S [17]. On the other hand, the decreased capacity of rhodanese, which is the key component in the H_2S removal system under low O_2 tensions, may effectively increase the H_2S concentrations [18]. Eto and colleagues [2] found that the excitatory neurotransmitter L-glutamate greatly enhanced the production of H_2S. And many studies have confirmed that there is a massive outpouring of extracellular glutamate after CA or cerebral ischemia and reperfusion injury [19, 20]. On the basis of these studies we hypothesize that hypoxia and glutamate excess drive increased post-ischemic H_2S levels and the synergistic increase with H_2S therapy.

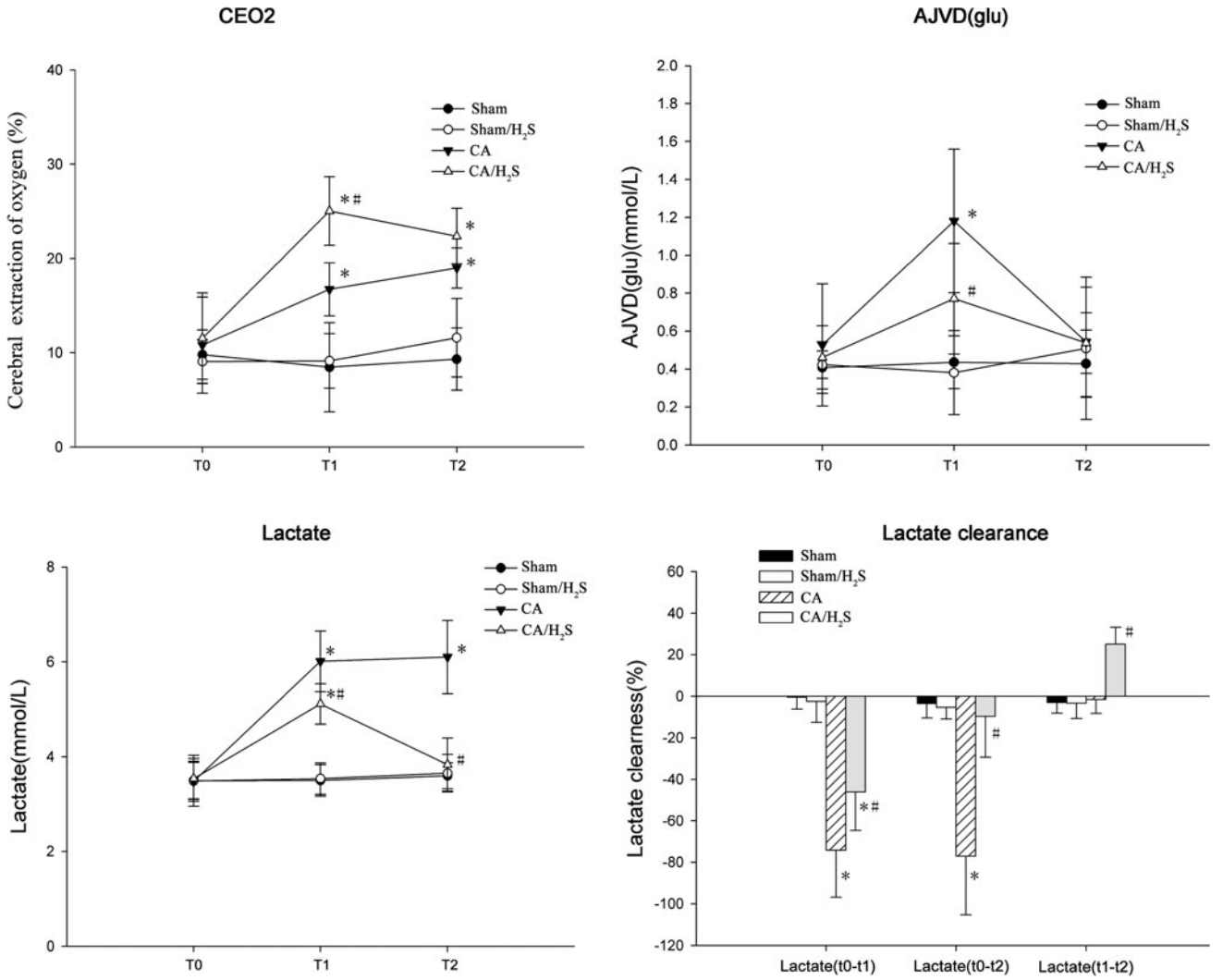

Fig. 6 Cerebral extraction of oxygen, arterio-jugular venous differences of glucose, and lactate in sham, sham/H_2S, CA, and CA/H_2S groups. Lactate clearance shows the clearance of lactate between t0 and t1 [*Lactate (t0–t1)*], between t0 and t2 [*Lactate (t0–t2)*], and between t1 and t2 [*Lactate (t1–t2)*] for each group. *T0*, *T1*, and *T2* represent baseline, 30 and 60 min after restoration of spontaneous circulation. $^{*}P < 0.05$ versus sham group. $^{\#}P < 0.05$ versus CA group

Much research has been published on the clinical utility of S100B in predicting neurological outcomes after CPR [21, 22]. In this study, we found that serum S100B levels were significantly increased after ROSC, whereas inhalation of H_2S significantly reduced the level of S100B, which predicted a better neurological outcome. This was confirmed by the results of NDS. The 24-h NDS data, when survivors were nearly equal between CA and CA/H_2S groups, clearly showed less neurological injury in H_2S treated animals. And this protective effect continued throughout the experiment, which was proven by the increase in viable neurons and survival at 7 days after ROSC. Taking all these factors together, the present data provide strong evidence that H_2S can improve early neurological outcomes after CA and resuscitation.

Although decreased body temperature and metabolism were observed in mice exposed to H_2S [1, 23], these results had been questioned in piglets [4], sheep [5], and even rats [6]. Similarly in our rabbit model, rectal, cortex, and hippocampus temperatures were not decreased significantly in H_2S treated animals. Owing to their large surface area/mass ratio, mice can experience rapid drops in core body temperature, whereas these are rare or impossible in larger animals. Another key factor was the low O_2 tension which was indispensable for H_2S-dependent hypometabolism [6]. Stein and colleagues [6] found that rats were largely insensitive to H_2S-induced low body temperature unless O_2 tension was decreased to 10.5 %. This finding was supported by our study with temperature results during the short periods of exposure to lowered O_2 tensions.

In the present study, we observed an increased CEO_2 after CA which is compatible with previous studies [24]. However, Lemiale and colleagues [25] found that CEO_2 reached nearly normal values over time in survivors. These different results of CEO_2 could probably be explained in part by the therapeutic hypothermia and midazolam used for sedation in Lemiale's study which can depress the brain metabolism. Another explanation could be the different degree of brain damage. In Lemiale's study, more severe brain damage was found in 10 of 18 patients and nearly half of them had serious seizures [25]. More severe brain damage meant extensive loss of viable neurons with a concomitant decrease in cerebral metabolism [24, 26].

The current findings also demonstrate that less glucose extraction and faster lactate clearance occur after inhalation of H_2S. Taking into account the higher CEO_2, one possibility could be a switch from anaerobic glycolysis induced by asphyxia to oxidative phosphorylations which consume more oxygen and less glucose while producing less lactate. In the latest study [27], evidence had shown that H_2S could correct the pathophysiological switch between oxidative phosphorylation and glycolysis, which gave a powerful support to our study.

Many studies demonstrated that the increased CEO_2 [25, 26] or lower $CJVO_2$ (content of oxygen in the jugular venous blood) [24, 28] and effective early lactate clearance were associated with improvement of neurological outcome and decreases in both early and late mortality in post-CA patients [29, 30]. Consistent with these findings, a better neurological outcome was confirmed with less S100B, more viable neurons, less NDS, and higher survival rate in H_2S treated animals which had increased CEO_2, decreased AJVD(glu), and enhanced lactate clearance.

This study has several limitations. Firstly, we used 80 ppm H_2S because this dose had been widely used in previous studies and no obvious neurotoxic effects were observed [1, 4, 31]. Although this will make it easier to draw comparisons, the relationship between H_2S concentration and favorable outcome has not been verified. Furthermore, the lack of measurement of CBF and ATP generation/ox-phos limits our ability to draw a conclusion whether the brain metabolism changes are related to delivery or consumption. Future studies are warranted to further investigate the relationship between H_2S effects and CBF and ATP generation/ox-phos. Lastly, though our findings demonstrate improvement of early neurological function after inhalation of 80 ppm H_2S in a rabbit CA model, the translational implication of H_2S in the human clinical setting is uncertain and needs to be investigated.

In summary, our study revealed that inhalation of 80 ppm H_2S reduced neurohistopathological damage and improved early neurological function after CA and resuscitation in rabbits. We suggest that improvements in cerebral metabolism evidenced by increasing CEO_2, decreasing AJVD(glu), and clearing lactate may be involved in the protective effects. A better understanding of the relationship between H_2S effects and CBF and ATP generation/ox-phos in future studies will be important in clarifying the underlying mechanisms.

Acknowledgments The study was supported by grants from the National Natural Science Foundation of China (81000822) and the Natural Science Foundation of Heilongjiang Province, Heilongjiang, China (QC2010056). This work was assisted in part by the Key Laboratory of Anesthesiology and Intensive Care Research of Heilongjiang Province, China.

Conflicts of interest There was no conflict of interest in this study.

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