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HYDROGEN SULFIDE DOES NOT INCREASE RESUSCITABILITY IN A PORCINE MODEL OF PROLONGED CARDIAC ARREST

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ABSTRACT—Treatment options to improve resuscitability and neurological prognosis after cardiac arrest (CA) are limited. Hydrogen sulfide has demonstrated remarkable improvements in outcomes in small animal models of severe hypoxia or hemorrhage. We investigated the influence of sodium sulfide (Na_2S), a liquid hydrogen sulfide donor, on resuscitability, postresuscitation hemodynamics, and neurological performance in a porcine model of prolonged CA and cardiopulmonary resuscitation. Twenty-four male pigs were instrumented with arterial and pulmonary artery catheters before 10 min of CA was induced. During resuscitation, animals were randomized to receive either high-dose (1 mg/kg; n = 8) or low-dose (0.3 mg/kg; n = 8) Na_2S (IK-1001; Ikaria, Clinton, NJ) or control (saline placebo; n = 8) i.v. injection and consecutive infusion. Cardiopulmonary resuscitation was performed for 6 min before defibrillation was attempted. Hemodynamic variables were taken at baseline and 10, 30, 60, 120, and 240 min after successful resuscitation. Neurological outcome was evaluated on 4 postoperative days before brains and hearts were harvested for histopathologic analysis. No differences in hemodynamic parameters were observed at baseline. Initial resuscitability was not improved by Na_2S. Animals exposed to high- and low-dose Na_2S showed significantly reduced cardiac output, heart rate, and pulmonary arterial pressure compared with control animals during the early postresuscitation period. Strikingly, two of the high-dose Na_2S animals died during the postresuscitation period, whereas all other animals survived. High-dose Na_2S significantly decreased microglial activation in striatal areas, although this did not translate into improved neurological outcome. Although animals receiving Na_2S developed higher troponin T serum levels, these differences remained insignificant. In this investigation, Na_2S did not improve resuscitability but significantly compromised postresuscitation hemodynamics.

KEYWORDS—Cardiac arrest, cardiopulmonary resuscitation, pigs, hydrogen sulfide, sodium sulfide, cerebral anoxia-ischemia

INTRODUCTION

Hydrogen sulfide (H_2S), a colorless gas with the typical odor of rotten eggs, has received considerably more attention because of its toxicity rather than its potential as a therapeutic agent. However, H_2S is synthesized by several tissues and involved in the regulation of a variety of physiological functions including cellular respiration, vasoactivity, inflammation, and neutrophil activation (1). Together with nitric oxide and carbon monoxide, H_2S is now recognized as the third endogenous gaseous transmitter. Administration of very low concentrations of exogenous H_2S exerted beneficial effects in clinically relevant models of hemorrhage, hypoxia, and I/R injury (2–5). Furthermore, promising results from investigations in neuronal cell cultures imply potential neuroprotective effects of the gas (6, 7).

Cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR) represents the most severe form of I/R injury. With more advanced technical equipment and clinical knowledge, the rate of return of spontaneous circulation has increased during the last decades. However, patients who are resuscitated from CA exhibit a distinct pathology termed *postresuscitation syndrome* (8). Clinically, this syndrome becomes mainly apparent as cerebral and myocardial dysfunction (9), both of which contribute to the substantial mortality in the early phase after successful CPR. Furthermore, a pronounced inflammatory response (10), alterations of the coagulation system (11), and inadequate adrenal response (12) have been described. Accordingly, the overall survival rate after successful CPR remains disappointingly low (13, 14). In a pioneering study, Blackstone and colleagues (15) demonstrated that H_2S induces a hibernation-like state in rodents and gave rise to the hope that the agent could protect cells and tissues from the fatal consequences of hypoxia and/or ischemia. This prompted us to investigate the effects of H_2S in a large animal model under the conditions of extreme hypoxia and ischemia followed by reperfusion.

Based on recent literature, we hypothesized that H_2S would increase initial resuscitability and ameliorate neurological damage. To test this hypothesis, we used an established large animal model that, in contrast to most rodent experiments, resembles a clinical situation.

MATERIALS AND METHODS

Subjects

In 24 male domestic pigs (*Sus scrofa*), ventricular fibrillation (VF) was induced, and CPR was performed as previously described (16–19). Animals weighing between 31 and 42 kg (35.9 ± 2.65 kg, mean ± SD) were supplied from a single source breeder and housed in a room that was adequately spaced and air-conditioned. A 12-h light-dark cycle from 6 AM to 6 PM was

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allowed. All procedures were in accordance to the Helsinki Declaration for the Use and Care of Animals and approved by the appropriate government institution (LANUV Recklinghausen, Recklinghausen, Germany; AZ: 9.93.2.10.35.07.276).

Neurocognitive testing

Five days before CPR and 4 days thereafter, animals had to perform a visuospatial memory task on the basis of operant conditioning (18). In a separated testing area, each pig had to obtain food from one of three wall-mounted troughs with movable lids. After familiarizing animals with these troughs, the lids of two containers were locked with a screw, leaving only one to be opened by the tested pig. Time from entering the area until food was obtained was recorded and registered every day before and after CPR, respectively.

Neurological evaluation

On each day of the observation period, animals were evaluated using a neurological deficit score (NDS) as previously described (18). Briefly, the score is composed of four items representing the level of consciousness, respiration, posture, and the ability to take food and water.

Animal preparation for CA and CPR

After i.m. premedication with 4 mg/kg azaperone, anesthesia was completed by ear vein injection of 15 mg/kg sodium pentobarbital. Animals were then intubated and mechanically ventilated (Sulla 808-V, Dräger AG, Lübeck, Germany) with a fraction of inspired O_2 of 21% and a tidal volume of 15 mL/kg. Respiratory frequency was adjusted to maintain end-tidal P_{CO_2} between 35 and 40 mmHg. No neuromuscular blockade was used in this investigation. For measurement of aortic pressure, a fluid-filled catheter (AKS-1830; CODAN pvb Medical, Forstinning, Germany) was advanced from the left femoral artery into the abdominal aorta. Cardiac output (CO) and blood temperature were continuously evaluated using a 7.5F pentalumen pulmonary artery catheter (744F75; Edwards Lifesciences, Irvine, Calif) advanced from the percutaneously punctured left femoral vein, flow-directed into the right ventricle. For induction of CA, a 5F pacing catheter was advanced from the surgically exposed left cephalic vein into the right ventricle. Blood temperature was maintained around 38.2°C ± 0.2°C throughout the experiment using a convective heating blanket (Warm Touch 5200; Tyco Healthcare, Pleasanton, Calif).

Experimental procedure

Thirty minutes before induction of CA, continuous infusion of pentobarbital (4 mg/kg per h) was stopped. Cardiac arrest was induced with 1 to 2 mA of alternating current delivered to the endocardium of the right ventricle, resulting in VF. Mechanical ventilation was discontinued at the same time. Ten minutes after onset of VF, precordial compression was started using a piston-driven chest compressor (Thumper 1007; Michigan Instruments, Grand Rapids, Mich) with 100 compressions/min, synchronized with the coincidentally restarted mechanical ventilation, to provide a compression-ventilation ratio of 30:2 with equal compression/relaxation interval (i.e., a 50% duty cycle) and a compression depth of 25% of the chest diameter. Ventilation was adjusted to deliver a tidal volume of 10 mL/kg and a fraction of inspired O_2 of 100%. Two bolus doses of 30 μg/kg epinephrine were injected i.v. after 1 and 4.5 min of precordial compression. Electrical defibrillation was attempted with up to two 150 J biphasic waveform shocks (M-Series CCT; Zoll Medical Corporation, Chelmsford, Mass) delivered between the right infraclavicular area and the cardiac apex. If an organized rhythm with mean aortic pressure of greater than 60 mmHg persisted for 5 min, the animal was regarded as successfully resuscitated. Failing to reverse VF, 1 min of chest compression and another bolus of 30 μg/kg epinephrine preceded delivery of another sequence of up to two shocks. If after five sequences VF persisted, resuscitation was stopped. In successfully resuscitated animals, measurements as later described were performed for 4 h. At the end of the experiment, all animals received i.m. injection of 0.1 mg/kg buprenorphine for pain relief and were weaned from the ventilator. After extubation, every animal was observed for 30 min to ensure adequate spontaneous breathing before being returned to its room.

Study drugs and randomization

Before the preparation period, each animal was randomized to one of three groups receiving either 1 mg (high dose) or 0.3 mg (low dose) per kg bodyweight of the liquid H_2S donor sodium sulfide ([Na_2S] IK-1001; Ikaria, Clinton, NJ) or an equivalent volume of 0.9% saline solution. Randomization and preparation of the appropriate medication were performed by a laboratory technician not involved in the study. Initial i.v. bolus doses as previously described were given 1 min after starting precordial compression and were immediately followed by continuous infusion of 1 mg/kg per h or 0.3 mg/kg per h of Na_2S or saline placebo for 2 h, according to the dosage chosen for the bolus injection.

Measurements

Gas exchange, hemodynamics, lactate and glucose levels were measured at baseline and in the postresuscitation period at 10, 30, 60, 120 and 240 min after return of spontaneous circulation. Arterial blood samples for the determination of troponin T serum levels were taken at identical time points and processed according to the manufacturer (Cobas e 411; Roche Diagnostics, Mannheim, Germany).

Histological evaluation

Brain tissue was harvested and evaluated as previously described (16–19). In brief, animals were anesthetized and mechanically ventilated on day 5 of the recovery period as previously described. After intravital perfusion with 4% formaldehyde, brains were carefully removed and stored for 2 weeks in the same fixative. Standardized coronal slices were taken at a thickness of 4 to 5 mm, resulting in a total of 14 to 15 slices. The anterior and posterior CA1 and CA3/4 sectors of the hippocampus, occipital neocortex, caudate nucleus, and putamen (striatum) were chosen as regions of interest (ROI) and paraffin embedded. Conventional hematoxylin and eosin staining was performed. Immunohistochemical reactions to visualize reactive astrogliosis (polyclonal rabbit anti-glial fibrillary acidic protein), microglial activation (monoclonal mouse CD68), and perivascular inflammatory response (monoclonal mouse myeloid/histiocyte antigen, monoclonal mouse CD45; all DakoCytomation Denmark A/S, Glostrup, Denmark) were also performed. An experienced neuropathologist (K.N.), blinded to the animals' treatment assignment, assessed histopathologic damage using a semiquantitative score as follows: for each ROI, the proportion of neurons with hypereosinophilia, shrunken cytoplasm, and pyknotic nuclei, indicative of ischemically induced necrotic damage, was graded (0%–10% = 1, 10%–20% = 2, 20%–50% = 3, 50%–80% = 4, 80%–100% = 5). In the well-defined hippocampal areas CA1 and CA3/4, additional analyses were performed by counting the ischemically damaged neurons and putting them in relation to the total amount of cells in this area. The extent of astrogliosis, microglial activation, and perivascular inflammation was also graded for each ROI as follows: absent = 0, mild = 1, moderate = 2, severe = 3.

To detect apoptotic cell changes in the hippocampal CA1 and CA3/4 sectors, TdT-mediated dUTP-biotin nick end labeling (TUNEL) staining was performed using the TdT-FragEL DNA fragmentation detection kit (Oncogene Research Products; Oncogene, Cambridge, Mass) as suggested by the manufacturer. The results were expressed as a percentage of TUNEL-positive cells in the whole cell population in this area.

The hearts of the killed animals were removed from the bodies shortly after intravital perfusion and stored in the same fluid for at least 3 weeks. After macroscopic demonstration of all chambers, small blocks of myocardial tissue were dissected from defined atrial as well as ventricular areas, embedded in paraffin, and cut into slices for further histopathologic examination. Besides conventional hematoxylin and eosin staining, slices were stained with von Kossa silver stain to visualize inorganic phosphate calcium salts.

Statistical analysis

Statistical analysis was performed using SPSS 14.0 data package for Windows (SPSS, Chicago, Ill). All data are reported as mean ± SD. For group comparisons of continuous variables at given time points, ANOVA was used and, in cases where significant differences were observed, was followed by paired Student *t* tests, adjusted for multiple comparisons (Bonferroni). Where appropriate, chi-square tests were performed to establish statistical differences for categorical variables. A $P \leq 0.05$ was regarded as statistically significant.

RESULTS

Of 24 animals, 13 could be successfully resuscitated. Initial survival was comparable between groups (four in the placebo group and four in the high-dose group versus five in the

TABLE 1. **Initial survival (return of spontaneous circulation) and after the observation period of 4 days**

	Return of spontaneous circulation	4 days
Control	4	4
1 mg/kg Na_2S	4	2
0.3 mg/kg Na_2S	5	5

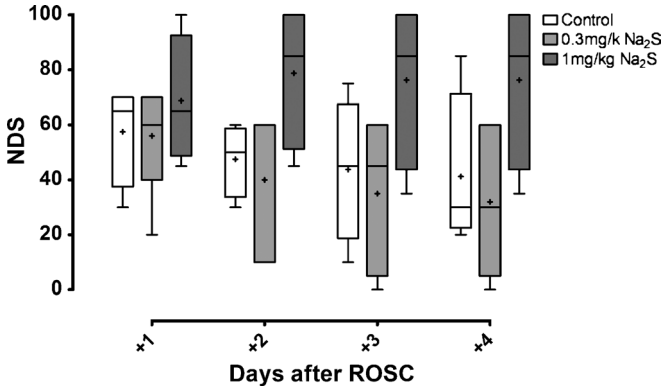

FIG. 1. **The NDS 4 days after CPR.** Zero equals normal cerebral performance, whereas 100 signifies brain death; +1 to +4, days 1 to 4 after CPR.

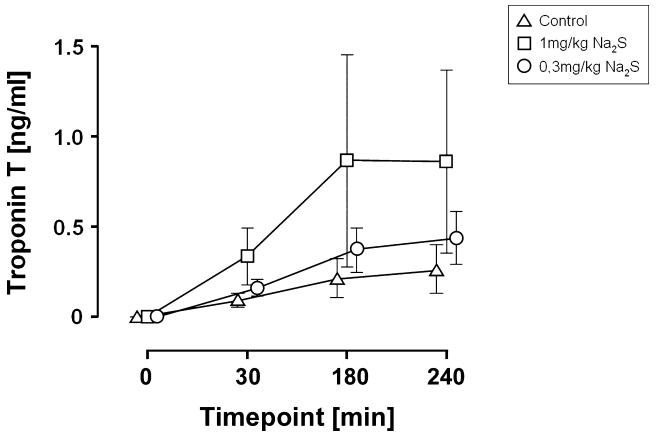

FIG. 3. **Troponin T serum levels in different treatment groups.** Measurements at 30, 180 and 240 min after return of spontaneous circulation.

low-dose group). Furthermore, no difference in CPR cycles needed to restore a sufficient circulation was observed. However, although all control and low-dose Na_2S animals survived until day 5, two animals treated with high-dose Na_2S prematurely died (Table 1).

As shown in Figure 1, animals receiving high-dose Na_2S performed worst on all days post-CPR with regard to the NDS, whereas low-dose animals exhibited a slightly better performance, however, with no significant differences between groups.

Hemodynamic variables including MAP, heart rate (HR), pulmonary capillary wedge pressure (PCWP), and CO did not differ between groups at baseline. Irrespective of the Na_2S dosage, MAP, pulmonary arterial pressure, CO, and PCWP were significantly lower in comparison to placebo-treated animals during the first 4 h after CPR. The reductions in CO and MAP were greatest during the first half hour post-CPR, reaching statistical significance 10 min (CO) and 30 min (MAP) post-CPR in animals treated with 1 mg/kg Na_2S (Fig. 2, A and B). Animals receiving Na_2S presented pronounced tachycardia, especially in the first 30 min post-resuscitation (Fig. 2C). Interestingly, PCWP was—especially in the high-dose group—significantly lower at all time points after CPR (Fig. 2D).

Decreased hemodynamic performance was accompanied by a marked increase in troponin serum levels that was, although not significant, more severe in animals treated with high-dose Na_2S (Fig. 3).

Besides P_{CO_2} and pH levels 30 min post-CPR, blood gases as well as lactate and glucose levels did not differ significantly between groups at baseline or in the postresuscitation period. There were also no differences in body temperature (Table 2).

Histological evaluation revealed severe ischemic brain lesions and gliosis in all groups. Except for a lesser degree of microgliosis, which was significantly reduced in the caudate nucleus and putamen in animals receiving high-dose Na_2S, there were no differences as a result of treatment (Fig. 4). The TUNEL assay revealed nameable apoptosis in hippocampal sections in two animals treated with 0.3 mg/kg Na_2S. In myocardial tissue, disseminated intracellular inclusions in two animals receiving 1 mg/kg Na_2S were observed in the right ventricle that predominantly consisted of calcium and phosphorus.

DISCUSSION

The present study primarily aimed to investigate the influence of Na_2S on resuscitability in the experimental setting of CA and CPR. Although Na_2S did not positively influence resuscitability, it had a measurable influence on postresuscitation hemodynamics. High-dose Na_2S treatment seemed to exert negative effects on survival.

Our work was prompted by the landmark studies of Blackstone and colleagues (2, 15) who demonstrated that very low doses of H_2S can induce a state of suspended animation in spontaneously breathing mice with no obvious toxic effects.

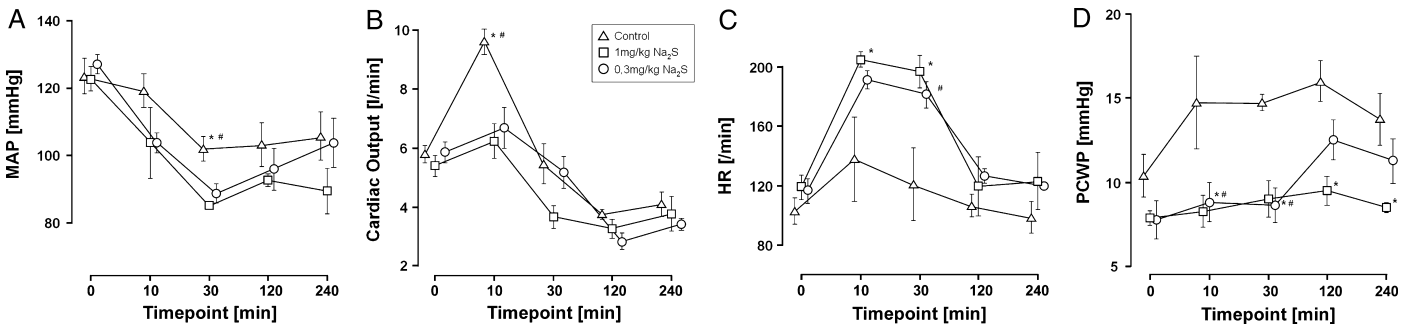

FIG. 2. **Hemodynamics**. A, MAP; B, CO; C, HR; D, PCWP. $^*P \leq 0.05$ control versus 1 mg/kg Na_2S; $^\#P \leq 0.05$ control versus 0.3 mg/kg Na_2S. Measurements at 10, 30, 120 and 240 min after return of spontaneous circulation.

TABLE 2. **Blood gas analyses and body temperature**

	Control	1 mg/kg Na_2S	0.3 mg/kg Na_2S	*P*
Baseline				
Pa_{O_2}, mmHg	129.6 ± 41.0	106.6 ± 10.0	115.1 ± 33.4	1.00
Pa_{CO_2}, mmHg	38.6 ± 4.1	37.2 ± 2.3	37.6 ± 2.5	0.24
Lactate, mg/dL	1.12 ± 0.38	1.24 ± 0.48	1.28 ± 0.48	0.57
Glucose, mmol/L	5.39 ± 1.00	5.66 ± 0.81	5.11 ± 0.55	1.00
pH	7.52 ± 0.05	7.53 ± 0.03	7.51 ± 0.03	1.00
Body temperature	38.2 ± 0.1	38.2 ± 0.2	38.1 ± 0.1	1.00
30 min post-CPR				
Pa_{O_2}, mmHg	417.5 ± 94.5	450.3 ± 43.4	431.6 ± 87.9	1.00
Pa_{CO_2}*, mmHg	37.6 ± 5.9	50.8 ± 7.1	43.5 ± 2.5	0.02
Lactate, mg/dL	5.73 ± 1.45	5.9 ± 1.40	7.68 ± 1.07	0.14
Glucose, mmol/L	12.80 ± 5.22	13.8 ± 3.90	12.38 ± 1.63	1.00
pH*	7.39 ± 0.00	7.31 ± 0.05	7.34 ± 0.03	0.02
Body temperature (°C)	37.9 ± 0.3	37.9 ± 0.2	37.9 ± 0.3	1.00
120 min post-CPR				
Pa_{O_2}, mmHg	134.5 ± 29.6	91.5 ± 6.7	128.7 ± 28.3	0.96
Pa_{CO_2}, mmHg	38.1 ± 3.2	42.0 ± 12.8	39.4 ± 1.8	1.00
Lactate, mg/dL	2.58 ± 1.16	3.55 ± 1.89	3.58 ± 0.89	0.87
Glucose, mmol/L	8.07 ± 1.67	10.00 ± 2.76	7.88 ± 1.54	0.45
pH	7.48 ± 0.02	7.44 ± 0.11	7.45 ± 0.03	0.92
Body temperature	38.1 ± 0.1	38.2 ± 0.2	38.2 ± 0.1	1.00
240 min post-CPR				
Pa_{O_2}, mmHg	125.6 ± 28.7	90.8 ± 6.5	125.1 ± 26.4	0.16
Pa_{CO_2}, mmHg	40.2 ± 2.4	43.1 ± 13.8	39.1 ± 3.1	1.00
Lactate, mg/dL	1.20 ± 0.30	1.45 ± 0.61	1.14 ± 0.27	0.84
Glucose, mmol/L	6.15 ± 1.01	7.23 ± 1.07	5.96 ± 1.01	0.29
pH	7.50 ± 0.01	7.45 ± 0.11	7.49 ± 0.04	1.00
Body temperature	38.1 ± 0.2	38.1 ± 0.1	38.2 ± 0.2	1.00

*$P \leq 0.05$ control versus 1 mg/kg Na_2S.

With the idea that H_2S may increase tissue viability and final outcome in life-threatening states of tissue hypoperfusion and I/R injury, several investigations already focused on its protective effects in animal models of sepsis (20, 21), myocardial infarction (22, 23), and stroke (7). These studies yielded contrasting results. Whereas rats exposed to lethal hemorrhage or hypoxia that received either inhaled H_2S or i.v. Na_2S showed improved survival (3), Li et al. (24) found that i.v. sodium hydrosulfide (NaHS) had a proinflammatory effect that aggravated sepsis-induced elevations in pulmonary and hepatic myeloperoxidase levels. In Langendorff-perfused hearts subjected to 30 min of ischemia followed by 120 min of reperfusion, NaHS resulted in a concentration-dependent limitation of infarct size. This effect was abolished when potent blockers of ATP-dependent potassium (K_{ATP}) channels were given, suggesting a possible role of K_{ATP} channels in the protective mechanism of H_2S (25). In a rodent model of myocardial infarction, pretreatment with NaHS significantly reduced mortality and infarct size (26). Less promising data have been found in cerebral ischemia. Although H_2S protects neuronal cell cultures from oxidative stress by increasing glutathione levels (7), conflicting results have been found in a rodent model of middle cerebral artery occlusion, in which pretreatment with H_2S translated in significantly larger infarct areas (27). Our results suggest that although high-dose Na_2S significantly decreased microglial activation in striatal areas (i.e., caudate nucleus and putamen), this did not translate in improved neurological recovery.

In general, H_2S seems to induce a hypometabolic response in rodent models that is inevitably followed by hypothermia (2, 15, 28). This circumstance has been primarily attributed to their large surface area–to–mass ratio and has been the focus of criticism because hypothermia might provide some degree of protection independent of the H_2S administration. Indeed, there are only little data on the metabolic effects of H_2S derived from large animal models. Li et al. (29) found that H_2S does not seem to show these hypometabolic effects in ambiently cooled piglets. In contrast, Simon and colleagues (4) demonstrated in a porcine model of aortic cross clamping that Na_2S administration leads to a significant reduction in core temperature that was accompanied by a lower O_2 uptake and CO_2 production. Furthermore, H_2S reduced the inherent hyperlactatemia (4). The results of our study suggest that in a whole-body model of I/R induced by CA and CPR, Na_2S does not reverse the profound lactatemia that is frequently observed in this model but aggravates acidosis and hypercapnia at least in animals treated with high-dose Na_2S

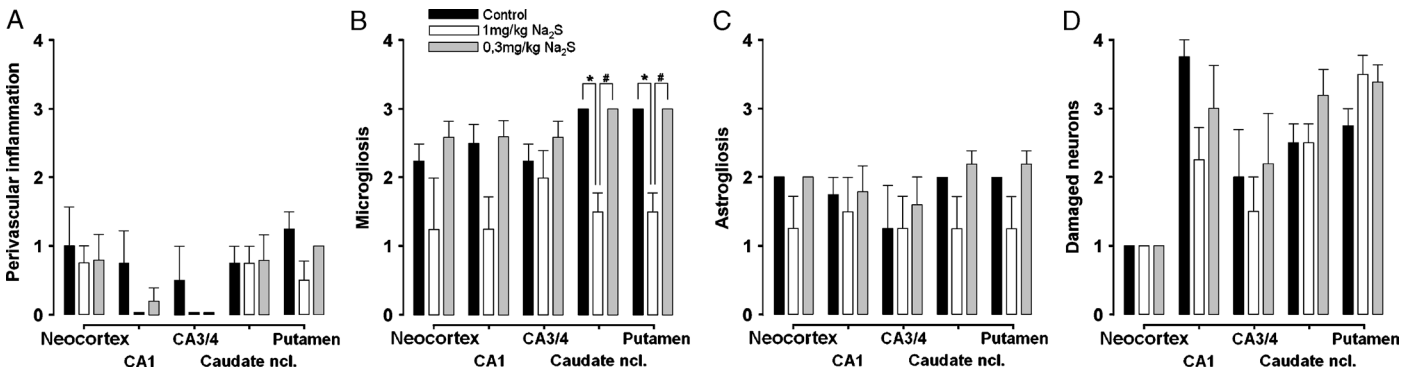

FIG. 4. **Histopathologic evaluation of all ROI in control and Na_2S-treated animals**. *$P \leq 0.05$ control versus 1 mg/kg Na_2S; #$P \leq 0.05$ 1 mg/kg Na_2S versus 0.3 mg/kg Na_2S. CA1, CA3/4, hippocampal sectors CA1, CA3/4; caudate ncl, caudate nucleus.

(Table 2). Furthermore, body temperature remained unchanged in our experiment.

Similar to nitric oxide and carbon monoxide, H_2S exerts several effects on the cardiovascular system. Hydrogen sulfide is a strong vasodilator and integral regulator of systemic and pulmonary blood pressure (30, 31). Indeed, application of Na_2S in the current study significantly decreased MAP 30 min post-CPR and PCWP throughout the whole postresuscitation period in animals receiving 1 mg/kg (Fig. 3). Unlike that seen in healthy animals (29) and most other models, Na_2S dramatically increased HR during the infusion period that was promptly reversed once the infusion was discontinued. Furthermore, PCWP values were significantly reduced, suggesting a decrease in left-sided filling pressures that would in turn explain the concomitant reductions in CO. These findings are in contrast to most of the studies on the protective effects of H_2S in myocardial infarction in which cardiac performance was mostly improved (5, 29).

We recognize certain limitations of this study. First, for safety reasons, it was decided not to block the olfactory sense of any personnel participating in the experimental procedure. Therefore, the intended double-blind randomization was hindered by the slight smell of rotten eggs that might have biased our results.

Second, the study was primarily designed to investigate the impact of Na_2S on resuscitability. Secondary data such as neurological function and hemodynamic changes must be interpreted with caution given the low numbers of surviving animals. Third, the results of our study are difficult to compare with previously published data in which the compound was almost exclusively given before the insult. Especially in the context of CA and CPR, other time points of administration might have yielded totally different results as has been recently demonstrated (32). However, to mimic a setting as close as possible to clinical reality, we decided to investigate an administration during rather than before the attempt of CPR, which depicts the earliest time point for professional interventions in the context of advanced life support. Finally, accumulation of Na_2S caused by low flow conditions during CPR might have led to hypercapnia and acidosis seen in both the high- and low-dose groups, which may have contributed to the adverse results seen in this investigation. Especially, the influence of hypercapnia and the assumed vasodilatory effects on vessels supplying cerebral tissues might have altered cerebral blood flow. Although desirable, the invasive measurement of cerebral blood flow would have precluded chronic studies and evaluation of neurological outcome. However, further investigations applying Na_2S in lower concentrations and/or at later time points are warranted to determine if the adverse effects seen in this study might also apply in different settings.

CONCLUSIONS

In conclusion, intra-arrest administration of two different doses of Na_2S did not reduce the high mortality observed in a porcine model of CA and CPR, and therefore, our initial hypothesis was disproven. However, postresuscitation hemodynamic function including MAP, CO, and PCWP were significantly reduced by the administration of Na_2S.

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