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Exogenous hydrogen sulfide produces hemodynamic effects by triggering central neuroregulatory mechanisms

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Recently, it was found that hydrogen sulfide (H_2S) may serve as an important transmitter in peripheral organs as well as in the brain. The aim of the present study was to evaluate the possible function of H_2S in the brain regulation of the circulatory system. Experiments were performed on conscious, male, Wistar-Kyoto rats. Mean arterial blood pressure (MABP) and heart rate (HR) were recorded continuously under baseline conditions and during infusions into the lateral cerebral ventricle (LCV) of the experimental animals. In control series LCV infusion of vehicle (Krebs-Henseleit bicarbonate-buffer) did not cause significant changes in MABP or HR. LCV infusion of H_2S donor (NaHS) at the rate of 400 nM/h resulted in an increase in MABP, whereas infusions at the rate of 100 nM/h and 200 nM/h failed to change MABP. On the other hand LCV infusion of H_2S donor at the rate of 200 nM/h caused a significant increase in HR while infusion at the rate of 400 nM/h produced an increase in HR, which was smaller than this observed during infusion at the rate of 200 nM/h. H_2S donor administered at the rate of 100 nM/h failed to affect HR. In conclusion, the present study demonstrates that exogenous hydrogen sulfide changes hemodynamic parameters by centrally mediated mechanisms. The hemodynamic effect seems to be dependent on H_2S concentration in cerebrospinal fluid. It appears that the hypertensive response may occur at a concentration, which does not exceed twice the physiological level.

Key words: hydrogen sulfide, blood pressure, heart rate, brain, rat

INTRODUCTION

Brain mechanisms have been shown to play important role in regulation of the circulatory system and several neurotransmitters and neuromodulators have been found to regulate hemodynamic parameters under physiological and pathophysiological conditions. Among gasotransmitters, nitric oxide (NO) has focused attention of investigators in particular. Although other gases, namely carbon monoxide (CO) and hydrogen sulfide (H_2S) have also been postulated to serve as transmitters in the brain, their role in central regulation of the cardiovascular system has not been determined yet.

Hydrogen sulfide is produced mainly from cysteine and its synthesis in the brain is performed by cystathionine-beta-synthetase (CBS). Interestingly, concentration of H_2S in the brain is significantly greater than that found in other organs (Abe and Kimura 1996, Zhao et al. 2001). It has also been suggested that pathological states such as stroke may be associated with overproduction of H_2S in the brain (Qu et al. 2006).

Recent studies by Zhao et al. showed that peripheral administration of H_2S donor (NaHS) results in decrease in blood pressure in anesthetized rats. The same investigators showed that the three-week-long treatment with inhibitors of cystathionine-gamma-lyase (CSE, enzyme synthesizing H_2S in peripheral tissues) increases systolic blood pressure in rats (Zhao et al. 2001, 2003). Further evidence for the importance of H_2S in regulation of hemodynamic parameters is provided by studies of Mok and colleagues (2004) who showed that inhibition of endogenous H_2S synthesis results in faster recovery of blood pressure in animals after hemorrhage. The hypotensive effect of H_2S seems to be pre-

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dominantly associated with vasorelaxant effect of H_2S, which is dependent on hyperpolarization of cellular membrane by means of opening ATP-dependent potassium channels (K_{ATP}), (Zhao et al. 2001, Cheng et al. 2004).

To our knowledge, there has been no evidence that hydrogen sulfide may influence hemodynamic parameters by means of the central neuroregulatory mechanisms. Therefore, the main goal of the present study was to evaluate the possible effect of H_2S on the brain regulation of the circulatory system.

METHODS

The experimental design, animal care and procedures were approved by the Ethical Committee of the Medical University of Warsaw.

Subjects

The experiments were performed on male, three-month-old Wistar Kyoto (WKY) rats, which were derived from different dams.

Reagents

The following drugs were used: Krebs-Henseleit bicarbonate-buffer, sodium hydrosulfide (NaHS; H_2S donor) and amiono-oxyacetate (AOO; H_2S synthesis inhibitor, inhibitor of cystathionine-beta-synthetase), (SIGMA-ALDRICH).

Procedures

Surgical preparation

All rats were chronically implanted with stainless steel cannula, which was inserted into the lateral cerebral ventricle (LCV) according to the following coordinates: 1.2 mm posterior to the bregma, 1.8 laterally to the midsaggital suture. The intracranial end of the cannula was located 4 mm below the skull surface. After the experiments the proper placement of the cannula was confirmed post mortem by administration of Evan's blue into the cerebroventricular system (only the animals with proper placement of the cannula into LCV were included in statistical analyzes). One week after cannula implantation a polyurethane arterial catheter was inserted through the femoral artery into the abdominal aorta. An intravascular part of the catheter was fixed 2.0 cm below the branching of the renal vessels. An extravascular part of the catheter was tunneled under the skin and exteriorized on the neck. Immediately after insertion of the catheter, the line was filled with a heparinized saline (100 units/ml) to prevent formation of a blood clot. All surgical procedures were performed under general anesthesia with ketamine 100 mg/kg i.p. After surgery the animals were given benzylathine penicyline 30 000 U i.m.

Measurements

Measurements were performed on freely moving rats 2 days after the arterial catheterization. In each series mean arterial pressure (MABP) was recorded on-line through the arterial catheter connected to the blood pressure recording system (Biopac MP100 unit). Heart rate (HR) was calculated from the consecutive systolic peaks on the blood pressure tracing by the AcqKnowledge v3.7 Biopac software.

The LCV infusions were performed using a microsyringe driven by the Harvard infusion pump. The solutions were LCV infused by means of a stainless steel infusion tube (ID 0.5 mm × OD 0.6 mm), which was inserted into the previously implanted cannula (ID 0.7 mm × OD 0.9 mm). The infusion tube was connected with a microsyringe by a polyethylene catheter.

Experimental series

The animals were randomly assigned to 9 experimental series. In all experimental series, after stabilization of hemodynamic parameters (on average 60 minutes), 10 min baseline measurement without any infusion were performed followed by 60 minute period of measurements during LCV infusions.

Control series

The buffering capacity of Krebs-Henseleit buffer, which was used as the vehicle for LCV infusion of NaHS (H_2S donor), was sufficient to maintain pH within the range of 7.5 up to the NaHS concentration of 10 mM. Therefore, in order to increase concentration of H_2S in cerebrospinal fluid (CSF) considerably above physiological level, the volumes of LCV infused vehicle had to be significantly higher than those tested in our previous studies. In order to establish the highest

volume/rate of LCV infusion, which does not influence hemodynamic parameters, four control series of experiments were performed. In series C1 (n=5), C2 (n=7), C3 (n=7), and C4 (n=5) the rats were infused with the vehicle at the rate of 10 µl/h, 20 µl/h, 40 µl/h and 80 µl/h, respectively.

LCV administration of H_2S donor (NaHS)

Intracerebroventricular infusions of NaHS solution in Krebs-Henseleit buffer (NaHS solution, 10 mM) were performed only at the rate tested in the control series C1, C2 and C3 since in the control series C4 the hemodynamic parameters were unstable during LCV infusion. Specifically, in series HS1 (n=5), HS2 (n=7) and HS3 (n=7) the rats were LCV infused with NaHS solution at the rate of 10 µl/h (100 nM of NaHS/h), 20 µl/h (200 nM of NaHS/h) and 40 µl/h (400 nM of NaHS/h), respectively. In order to exclude peripheral origin of an effect observed during LCV infusion of NaHS, we carried out additional experiments in which rats were intravenously infused with NaHS solution at the rate, which was found to produce hemodynamic changes during LCV infusions (HS2 and HS3 series).

LCV administration of H_2S synthesis inhibitor (cystathionine-beta-synthetase inhibitor, AOO)

The effect of LCV infusion of AOO solution in the Krebs-Henseleit buffer (10 mM) was tested in two experimental series. In series AO1 (n=5) and AO2 (n=5) the LCV infusions were performed at the rate of 20 µl/h (200 nM of AOO/h) and 40 µl/h (400 nM of AOO/h), respectively.

Statistics

The results are expressed as means and standard error (S.E.M.). For comparisons within the same series averaged measurements of 5 min baseline period preceding the LCV infusion and the consecutive 5 min periods during infusion were analyzed with one-way ANOVA for repeated measurements followed by Tukey's multiple range test. The differences in changes in MABP and HR between various treatments were evaluated by two-way ANOVA for repeated measurements on differences from the preinjection level. The multiple t-test with Bonferroni adjustment was used to isolate significant difference between individual means only when ANOVA comparisons were significant (P<0.05) (Ludbrook 1994).

RESULTS

Baseline MABP and HR in all the experimental series were similar (Table I).

Control series

In the control series of experiments C1, C2 and C3, LCV infusion of vehicle did not cause significant difference in either MABP or HR from baseline (Figs 1b and 2b).

The effect of LCV administration of H_2S donor (NaHS)

In the experimental series HS1 and HS2, LCV infusion of NaHS solution did not cause significant changes in MABP from baseline, whereas in series HS3 significant increase in MABP occurred after 30 min

Table I

Mean arterial blood pressure (MABP) and heart rate (HR) under baseline conditions in WKY rats

Series	C 1	C 2	C 3	HS 1	HS 2	HS 3	AO 1	AO 2
MABP (mmHg)	118.6 ± 2.2	117.8 ± 2.2	115.1 ± 1.7	116.4 ± 2.9	115.3 ± 2.5	116.4 ± 2.2	117.5 ± 2.1	116.3 ± 2.5
HR (beats/min)	331.1 ± 17.1	344.7 ± 7.1	359.8 ± 10.9	352.0 ± 14.2	339.8 ± 10.4	334.0 ± 9.9	346.5 ± 7.9	356.1 ± 13.1

Means ± S.E.M.

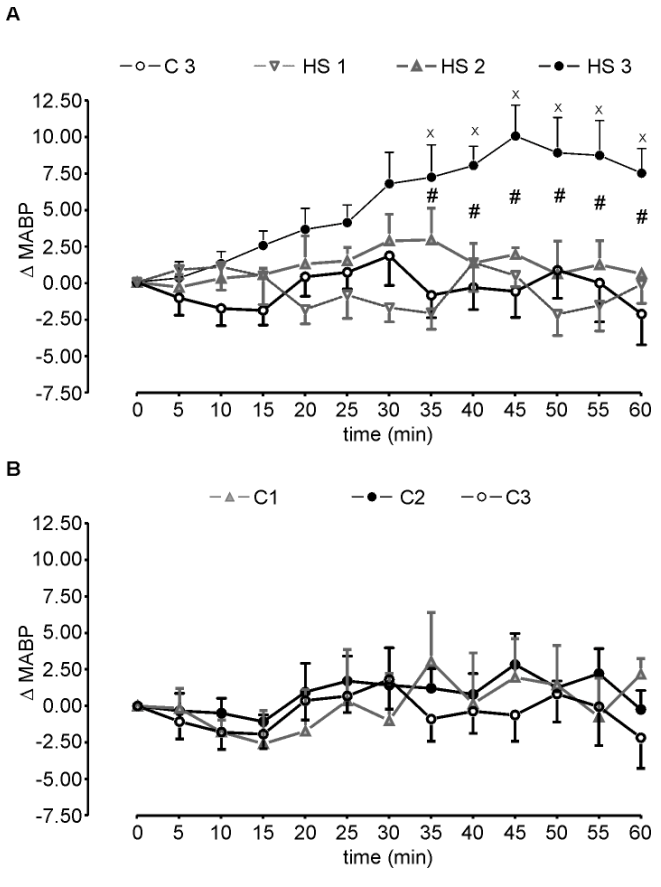

Fig. 1. Changes in mean arterial blood pressure (MABP, mmHg) in WKY rats during intracerebroventriclular (LCV) infusion. Averaged data for 5 min intervals are shown. (a) LCV infusions of: vehicle (Series C3, *n*=7), NaHS at the rate of 100 nM/h (series HS1, *n*=5), NaHS at the rate of 200 nM/h (series HS2, *n*=7) and NaHS at the rate of 400 nM/h (series HS3, *n*=7). (x) $P<0.05$ vs. baseline (series HS3), by Tukey's test; (#) $P<0.05$ series HS3 vs. series C3, by multiple *t*-test. (b) LCV infusions of vehicle at the rate: 10 µl/h (series C1, *n*=5), 20 µl/h (series C2, *n*=7) and 40 µl/h (series C3, *n*=7).

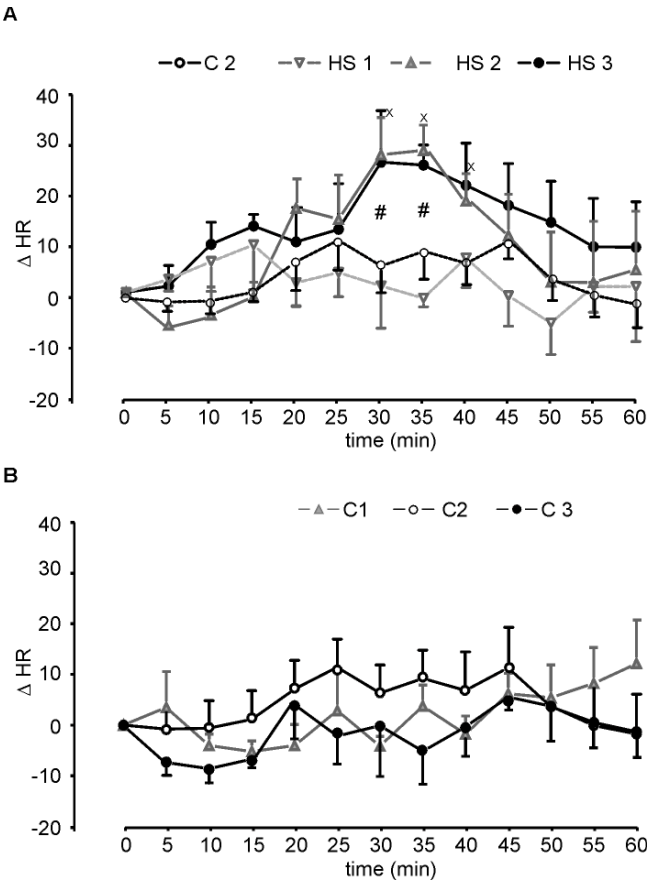

Fig. 2. Changes in heart rate (HR, beats/min) in WKY rats during intracerebroventriclular (LCV) infusion. Averaged data for 5 min intervals are shown. (a) LCV infusions of: vehicle (Series C2, *n*=7), NaHS at the rate of 100 nM/h (series HS1, *n*=5), NaHS at the rate of 200 nM/h (series HS2, *n*=7) and NaHS at the rate of 400 nM/h (series HS3, *n*=7). (x) $P<0.05$ vs. baseline (series HS2), by Tukey's test; (#) $P<0.05$ series HS2 vs. series C2, by multiple *t*-test. (b) LCV infusions of vehicle at the rate: 10 µl/h (series C1, *n*=5), 20 µL/h (series C2, *n*=7) and 40 µL/h (series C3, *n*=7).

of LCV infusion and lasted until the end of the experiment ($F_{12,72}$=4.11, $P<0.001$, one-way ANOVA), (Fig. 1a). Two-way ANOVA revealed significant differences in changes in MABP between HS3 series and C3 series ($F_{1,12}$= 18.5, $P<0.001$). Administration of NaHS in HS2 series produced significant increase in HR from baseline ($F_{12,72}$=3.40, $P<0.001$, one-way ANOVA), (Fig. 2a). On the other hand in HS3 series, statistical analysis with one-way ANOVA revealed that the increase in HR was not significant [HS3 series ($F_{12,72}$=1.79, $P<0.067$)]. Heart rate changes in HS2 series and HS3 series were significantly greater than those found in C2 and C3 series [HS2 series vs. C2 series ($F_{1,4}$=4.87, $P<0.05$), HS3 series vs. C3 series ($F_{1,4}$ =4.82, $P<0.05$), respectively, two-way ANOVA, analysis of periods between 30 and 50 min of LCV infusion] (Fig. 2a). Intravenous infusion of the NaHS solution at the same rate as in HS2 and HS3 series did not case significant changes in MABP and HR.

The effect of LCV administration of the inhibitor of endogenous H_2S synthesis (AOO)

Neither in AO1 series nor in AO2 series LCV infusion of AOO produced significant changes in MABP and HR from baseline.

DISCUSSION

The new finding of the present study is that exogenous hydrogen sulfide affects hemodynamic parameters by means of brain mechanisms concerned with regulation of the circulatory system. It appears that the hemodynamic response may result from the increase in H_2S concentration in CSF, which does not exceed twice the physiological concentration level.

A number of neurotransmitters and neuromodulators have been found to have an effect on the activity of brain neurons, which regulate the hemodynamic parameters by controlling the operation of the autonomic nervous system, synthesis and release of various hormones (Dampney 1994). Recently, it was found that hydrogen sulfide may serve as an important transmitter in peripheral organs as well as in the brain (Dello et al. 2000, Kimura 2002, Geng et al. 2004, Dombkowski et al. 2005, Han et al. 2005, Kimura et al. 2006). In the brain synthesis of H_2S is performed mainly by cystathionine-beta-synthetase (CBS) whereas in peripheral tissues cystathionine-gamma-lyase (CSE) seems to play more important role (Zhao et al. 2003, Chen et al. 2004, Kamoun 2004). Intriguingly, the concentration of H_2S in the brain reaches the level of 150–200 μM and therefore is significantly higher than in other tissues (Abe and Kimura 1996, Zhao et al. 2001). In this line it is worth mentioning that pathological states including stroke and septic shock seem to be associated with overproduction of the gas (Hui et al. 2003, Collin et al. 2005, Li et al. 2005, Qu et al. 2006). Furthermore, because of relatively long half-life of H_2S (minutes) and its high solubility in lipophilic solvents (Wang 2002), it is possible that peripherally released H_2S may act on tissues, which are distant form the place of its release, including the brain.

Accumulating data suggest that H_2S plays important role in regulation of the circulatory system. For instance, it has been shown that peripheral administration of hydrogen sulfide donor (NaHS) results in short lasting hypotensive effect (Zhao et al. 2001). The opposite effect i.e. increase in blood pressure was found in rats chronically treaded with CSE inhibitors (Zhao et al. 2003, Yan et al. 2004). Moreover, Mok and coauthors (2004) showed that inhibition of endogenous H_2S synthesis results in faster recovery of blood pressure in animals after hemorrhage. All these effects seem to be associated with direct vasorelaxant effect of H_2S (Zhao et al. 2001, Cheng et al. 2004). Additionally, recent studies by Xiao and others (2006), who found that H_2S increases sensitivity of carotid baroreflex in rats, provide an indirect evidence for possible role of the gas in neural regulation of the circulatory system.

In our experiments LCV infusion of H_2S donor produced both increase in MABP and HR. Interestingly, the changes in HR appeared during infusion of the lower dose of H_2S donor (series HS2), which at the same time failed to affect MABP. On the other hand, the higher dose (series HS3) produced significant increase in MABP but the changes in HR were smaller than those observed during infusion of the lower dose. Therefore, we believe that the primary action of the lower dose of H_2S is an acceleration of the heart rate, whereas the higher dose of H_2S may also produce increase in arterial blood pressure. The pressor effect might in turn trigger the baroreflex and likely leads to the decline in HR. As mentioned above, H_2S was shown to facilitate the carotid sinus barorefelx in rats (Xiao et al. 2006), and in this way may prevent an acceleration of the heart rate.

We hypothesize that there are at least two pathways, which may be involved in the hemodynamic changes during LCV infusion of H_2S donor. One possible explanation is that H_2S may directly act on neurons concerned with regulation of the circulatory system. However, the other hypothesis which involves an interaction between H_2S and nitric oxide in the brain seems to be likely as well. Several lines of evidence suggest significant role of such interaction in peripheral tissues. Specifically, Ali and coworkers (2006) showed that H_2S may inhibit vasorelaxant effect of NO, presumably by forming molecule (possibly nitrosothiol), which shows little or no vasorelaxant activity. Additionally, Kubo and others (2007) showed that H_2S may inhibit endothelial nitric oxide synthesis. It has been shown repeatedly that central infusion of NO donors decreases blood pressure and/or heart rate, whereas infusion of inhibitors of NO synthesis produces the opposite effect (Nurminen et al. 1997, Ufnal et al. 2006). Therefore, the hemodynamic effect of H_2S can result from inhibition of NO action or its synthesis.

In the present study the hemodynamic changes resulted from an increase in H_2S concentration in CSF to the level, which seems to be about twice of that found under physiological conditions. This conclusion is based on the following assumptions. First, volume of CSF in rats averages 250 μl. The concentration of H_2S in CSF

should not exceed 200 μM, because it has been shown that concentration of H_2S in the brain and plasma is within the range of 45 μM and 200 μM (Abe and Kimura 1996, Zhao et al. 2001). Second, molecular form of H_2S, which is postulated to be the active species, constitutes approximately 20% of H_2S in the NaHS solution [calculated for: pH 7.4; temperature 37°C, pK = 6.755 (Dorman et al. 2002)]. Finally, the changes in blood pressure occurred after 30 minutes of the infusion (i.e. administration of 200 nM of NaHS). The calculation does not take into account a degradation of H_2S in CSF, though. This may be a potential source of an overestimation of H_2S concentration in the brain, which is sufficient to affect hemodynamic parameters. On the other hand, because of the localization of intracranial end of the infusing tube, the concentration of H_2S in cerebrospinal fluid in the proximity of circumventricular organs could reach significantly higher values.

It is well known that changes in pH of the CSF may trigger central cardiovascular reflexes and may consequently evoke hemodynamic effects. The methodological challenge of the study was to administer an amount of the H_2S, which can trigger hemodynamic changes, and at the same time does not disturb pH of CSF. The pH of NaHS solution in the Krebs-Henseleit buffer is relatively stable (pH =7.5) only up to NaHS concentration of 10mM. Therefore, in order to increase the baseline values of H_2S concentration in CSF to the level, which has an effect on hemodynamic parameters we were forced to administer considerably high volumes of the vehicle into the cerebroventricular system of the experimental animals. Hence, in the control series (Series C3) we established the highest volume of the vehicle, which does not influence the cardiovascular parameters by affecting intracranial pressure. Yet the effort to avoid changes in pH of CSF while administering exogenous H_2S might not be necessary to reflect conditions in which increased synthesis of endogenous H_2S is present. Arguably, under such circumstances increased synthesis of H_2S may be accompanied by simultaneous changes in pH of cerebrospinal fluid.

The lack of hemodynamic changes during LCV infusion of AOO may lead to the conclusion that under baseline conditions H_2S transmission does not play an important role in the central regulation of the circulatory system or that decrease in H_2S transmission is compensated by activity of some other neurotransmitter pathways. However, it is also possible that in our experiments LCV infusion of AOO failed to inhibit H_2S synthesis in the brains of the experimental animals. Although, the concentrations of AOO solution, which was used in our study was shown to be sufficient to inhibit H_2S production by more than 70% in the brain under *in vitro* conditions it may fail to act in the same manner *in vivo*. Observations with regard to the differences of AOO inhibitory potential under *in vivo* vs. *in vitro* conditions were made by the Zhao and coworkers (2003) and Abe and Kimura (1996). It is possible that the lack of inhibitory effect of AOO may result from the weak cell membrane permeability for the compound. Further studies involving treatment with some newer specific CBS inhibitors, which could easily cross the cell membrane (to our knowledge such compounds are not available commercially yet) may be needed to clarify the role of H_2S in central regulation of the circulatory system under baseline conditions.

CONCLUSION

The present study demonstrates that exogenous hydrogen sulfide changes hemodynamic parameters by centrally mediated effects. It appears that the central pressor response may occur at a concentration of H_2S in cerebrospinal fluid, which does not exceed twice the physiological level. Therefore H_2S may play a significant role in central neuroregulation of the cardiovascular system under pathological conditions such as stroke or septic shock when increased synthesis of H_2S is present.

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