Pharmacological Reports 66 (2014) 751–758

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Exogenous hydrogen sulfide causes different hemodynamic effects in normotensive and hypertensive rats via neurogenic mechanisms

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ARTICLE INFO

Article history:
Received 2 May 2013
Received in revised form 7 April 2014
Accepted 8 April 2014
Available online 28 April 2014

Keywords:
Angiotensin II
Blood pressure
Hydrogen sulphide
Hypertension
Brain

ABSTRACT

Background: Increasing evidence suggests that disturbances in H_2S homeostasis may participate in the development of hypertension. In this study we compared hemodynamic responses to intracerebroventricular (ICV) infusions of sodium hydrosulfide (NaHS), a H_2S donor, between normotensive rats (WKY), spontaneously hypertensive rats (SHR) and angiotensin II – induced hypertensive rats (WKY-Ang II).
Methods: We tested the effects of NaHS on mean arterial blood pressure (MABP) and heart rate (HR) in 12–14-week-old, male rats. MABP and HR were continuously recorded at baseline and during ICV infusion of either vehicle (Krebs–Henseleit buffer) or NaHS.
Results: ICV infusions of the vehicle did not affect MABP and HR. WKY rats infused with 30 nmol/h of NaHS showed a mild decrease in MABP and HR. ICV infusion of 100 nmol/h produced a biphasic response i.e. mild hypotension and bradycardia followed by an increase in MABP and HR, whereas, the infusion of 300 nmol/h of the H_2S donor caused a monophasic increases in MABP and HR. In contrast, SHR rats as well as WKY-Ang II rats showed a decrease in MABP and HR during ICV infusions of NaHS.
Conclusions: The results provide further evidence for the involvement of H_2S in the neurogenic regulation of the circulatory system and suggest that alterations in H_2S signaling in the brain could be associated with hypertension.

Introduction

The toxic effects of exposure to high concentrations of hydrogen sulfide (H_2S) have been known for many centuries. However, only over the last two decades have the physiological functions of H_2S as gaseous mediator been extensively investigated. This was triggered by Abe and Kimura describing the enzymatic production of H_2S in the brain and showing its ability to influence long-term potentiation [1]. A growing body of data suggests that H_2S is an important biological mediator involved in various physiological processes [2,3]. Recently, several H_2S releasing compounds were developed; and their pharmacotherapeutic potential in cardiovascular, gastrointestinal and immunological diseases are being tested [4].

In the circulatory system H_2S has been found to affect vascular tone [5,6] and cardiac functions [7–10]. Several studies have shown that peripherally administered H_2S donors produce hypotensive effect [11,12]. It seems, however, that the effects of H_2S in the circulatory system depend on the dose as well as animal species [9,13]. In this context, H_2S has been found to dilate mesenteric [14] and hepatic [15] arteries in rats as well as human corpus cavernosum [16] but contract rat and duck aorta [13]. Furthermore, it has been reported that H_2S contracts rat and mouse aorta and human arteries at 10–100 μM concentrations while relaxes these vessels at 100–1000 μM concentrations [17–19]. Similarly, H_2S may exert both positive as well as negative chronotropic effect on the heart [9,10].

It has been postulated that decreased synthesis of endogenous H_2S plays a role in the development of hypertension. Yang and collaborators found that genetic deletion of cystathionine gamma-lyase (CSE), an enzyme which generates H_2S, produces hypertension in mice [20]. Furthermore, it has been found that peripheral administration of H_2S donors and precursors decreases blood pressure in several experimental models of hypertension such as hypertension induced by chronic inhibition of nitric oxide synthase, two-kidney-one-clip and in spontaneously hypertensive rats (SHR) [21–23].

Accumulating evidence highlights the crucial role of cardiovascular centers in the brain regulation of the circulatory system [24]. An increasing number of mediators, including gaseous

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http://dx.doi.org/10.1016/j.pharep.2014.04.004
1734-1140/

transmitters are being added to the group of mediators involved in the neurogenic control of arterial blood pressure [25]. In our previous studies we found that intracerebroventricular (ICV) infusions of NaHS, a H_2S donor exerts significant hemodynamic effect in normotensive WKY rats [9], which imply that H_2S plays a role in the brain control of arterial blood pressure.

In this study we compared hemodynamic responses to ICV infusions of NaHS between normotensive rats (WKY), spontaneously hypertensive rats (SHR) and WKY rats with angiotensin II (Ang II) – induced hypertension.

Materials and methods

The study was carried out in accordance with domestic and European Union guidelines of animal welfare. The experimental design, animal care and procedures were approved by the Second Local Animal Research Ethics Committee at the Medical University of Warsaw.

Animals

We performed the experiments on 12–14 weeks old, conscious male Wistar Kyoto (WKY) and Spontaneous Hypertensive (SHR) rats.

Drugs

The following drugs were used: Hexamethonium (Hexamethonium bromide, Sigma–Aldrich, Switzerland), Angiotensin II (Sigma–Aldrich, USA), Krebs–Henseleit bicarbonate-buffer (Sigma–Aldrich, USA), sodium hydrosulfide (NaHS; Sigma–Aldrich, Germany). NaHS was used as a H_2S donor and was prepared 10 min. before the onset of ICV infusion. Due to limited buffering capacity of the buffer, in order to maintain pH 7.45–7.5 the maximum 10 mM NaHS was prepared. Administration of increasing doses of NaHS was achieved by infusing increasing volumes of NaHS solution at a rate of 3–30 μl/h.

Surgical preparation

All rats were implanted with a stainless steel cannula into the lateral cerebral ventricle and week later an arterial catheter was inserted through the femoral artery into the abdominal aorta as previously described [9]. All surgical procedures were performed under general anesthesia with ketamine 100 mg/kg of bw *ip* (Bioketan, Vetoquinol Biowet, Poland) and xylazine 10 mg/kg of bw *ip* (Xylapan, Vetoquinol Biowet, Poland). After surgery the animals were given benzathine penicillin 30 000 IU IM (Debecylina, Polfa Tarchomin, Poland).

Hemodynamic measurements

Measurements were performed 2 days after the second surgery. Mean arterial blood pressure (MABP) was recorded on-line through the arterial catheter connected to the blood pressure recording system (Biopac MP100 unit; Biopac Systems, Goleta, CA). All measurements were done in animals freely moving in their living cages after stabilization of hemodynamic parameters (45 ± 15 min). MABP and HR were recorded at baseline (10 min) and during ICV infusions (45 min). Heart rate (HR) was calculated from the consecutive systolic peaks on the blood pressure tracing by the AcqKnowledge v3.7 Biopac software. ICV infusions were performed through a stainless steel infusion tube which was inserted into the previously implanted cannula.

Experimental series

WKY and SHR rats were randomly assigned to experimental series. In control series normotensive WKY rats (WKY-Control; $n = 6$) and hypertensive SHR rats (SHR-Control; $n = 6$) were infused with the Krebs–Henseleit buffer at a rate of 30 μl/h. ICV infusions of 10 mM NaHS was administered to WKY and SHR rats either at a dose of 30 nmol/h (WKY-30_{H_2S} series, $n = 6$ and SHR-30_{H_2S} series, $n = 6$), 100 nmol/h (WKY-100_{H_2S} series, $n = 6$ and SHR-100_{H_2S} series, $n = 6$) or 300 nmol/h (WKY-300_{H_2S} series, $n = 6$ and SHR-300_{H_2S} series, $n = 6$). Additionally, to prove that hemodynamic effects of ICV infused NaHS are mediated by the brain mechanisms and not by peripheral action of NaHS, we did the two series of experiments. In the first experiment WKY and SHR rats were concomitantly infused ICV with 300 nmol/h of NaHS and IV with hexamethonium, a ganglionic blocker (10 mg bolus followed by continuous infusion at the dose of 0.5 mg/min/rat, WKY-300_{H_2S+Hex} series, $n = 6$ and SHR-300_{H_2S+Hex} series, $n = 6$, respectively). In the second experiment WKY and SHR rats were infused IV with 300 nmol/h of NaHS.

Finally, to compare the pattern of hemodynamic response to NaHS in two different animal models of hypertension, we checked the response to ICV infusion of either: 300 nmol/h of NaHS or the Krebs–Henseleit buffer at a rate of 30 μl/h in SHR rats (SHR-300_{H_2S} series, $n = 6$ and SHR-Control series, $n = 6$) and in WKY rats with hypertension induced by Ang II (WKY-Ang II-300_{H_2S} series, $n = 6$ and WKY-Ang II-Control series, $n = 5$). Hypertension in WKY rats was induced by chronic infusion of Ang II at 450 ng/1 min/kg bw for 10 days, using a subcutaneously implanted osmotic minipump (Alzet 2ML2).

Data analysis

The results are expressed as the mean ± SEM. For the evaluation of the effects of ICV infusions within series, the average over 5 min before the infusion was compared with the average over 5 min after the onset of infusion by one-way repeated-measures analysis of variance (ANOVA), followed by Tukey's test. Comparisons between the series were evaluated by one- or two-way ANOVA for repeated measures when appropriate, followed by Tukey's test. Two-sided $p < 0.05$ was considered significant. All analyses were conducted using STATISTICA 6.0 (StatSoft, Krakow, Poland).

Results

Baseline MABP was significantly higher in SHR and WKY-Ang II groups than in WKY group. There were no significant differences in MABP and HR between series within the groups (Table 1).

WKY group

In WKY-Control series ICV infusions of the buffer did not affect MABP and HR (Fig. 1A and B). ICV infusion of NaHS in WKY-30_{H_2S} series decreased MABP [$F(1,9) = 36.5$, $p < 0.001$] and HR [$F(1,9) = 15.6$, $p < 0.001$] (Fig. 1A, 1B). Infusion of H_2S donor in WKY-100_{H_2S} series produced biphasic response, i.e. decrease in MABP and HR for the first 20 min of ICV infusion [$F(1,4) = 10.6$, $p < 0.001$ and $F(1,4) = 6.7$, $p < 0.001$, for MABP and HR respectively], followed by a significant increase in MABP and HR [$F(1,5) = 7.8$, $p < 0.001$ and $F(1,5) = 7.5$, $p < 0.001$, for MABP and HR respectively]. In WKY-300_{H_2S} series we found significant increase in MABP and HR [$F(1,9) = 34.7$, $p < 0.001$ and $F(1,9) = 28.3$, $p < 0.001$, for MABP and HR respectively] (Fig. 1A and B). In WKY-300_{H_2S+Hex} series, ganglionic blockade with hexamethonium produced a significant decrease in MABP (mmHg) from 114.3 ± 0.5 to 67.2 ± 0.4 and HR (beats/min) from 329 ± 2 to 250 ± 3. The following ICV infusions of 300 nmol/h of NaHS did not affect MABP and HR