

Captopril Partially Decreases the Effect of H₂S on Rat Blood Pressure and Inhibits H₂S-Induced Nitric Oxide Release From S-Nitrosoglutathione

M. DROBNÁ¹, A. MISAK², T. HOLLAND², F. KRISTEK¹, M. GRMAN^{2,3}, L. TOMASOVA^{2,4},
A. BERENYIOVA¹, S. CACANYIOVA¹, K. ONDRIAS^{2,3}

¹Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Bratislava, Slovak Republic, ²Institute of Molecular Physiology and Genetics, Slovak Academy of Sciences, Bratislava, Slovak Republic, ³Center for Molecular Medicine, Slovak Academy of Sciences, Bratislava, Slovak Republic, ⁴Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic

Received March 6, 2014

Accepted September 15, 2014

On-line December 3, 2014

Summary

We studied the effects of the H₂S donor Na₂S on the mean arterial blood pressure (MAP) and heart and breathing rates of anesthetized Wistar rats in the presence and absence of captopril. Bolus administration of Na₂S (1-4 μmol/kg) into the right jugular vein transiently decreased heart and increased breathing rates; at 8-30 μmol/kg, Na₂S had a biphasic effect, transiently decreasing and increasing MAP, while transiently decreasing heart rate and increasing and decreasing breathing rate. These results may indicate independent mechanisms by which H₂S influences MAP and heart and breathing rates. The effect of Na₂S in decreasing MAP was less pronounced in the presence of captopril (2 μmol/l), which may indicate that the renin-angiotensin system is partially involved in the Na₂S effect. Captopril decreased H₂S-induced NO release from S-nitrosoglutathione, which may be related to some biological activities of H₂S. These results contribute to the understanding of the effects of H₂S on the cardiovascular system.

Key words

H₂S • Nitric oxide • Captopril • Blood pressure • Heart rate

Corresponding author

K. Ondrias, Center for Molecular Medicine, Slovak Academy of Sciences, Vlarska 3-7, 831 01 Bratislava, Slovak Republic.
Fax: +421-2-54773666. E-mail: karol.ondrias@savba.sk

Introduction

The H₂S endogenously produced in mammals affects many of their physiological and pathological functions including the modulation of the NO signaling pathway and cardiovascular system. H₂S has been reported to influence vascular smooth muscle, myocardial ischemia, ischemia/reperfusion injury, pre-conditioning and post-conditioning (reviewed in Liu *et al.* 2012, Tomaskova *et al.* 2011, Wang 2012). These findings are currently facilitating detailed research into the molecular mechanisms of H₂S interactions to exploit its positive effects in the treatment of several diseases (Predmore and Lefer 2010).

Captopril (CAP), an angiotensin-converting enzyme inhibitor that influences cardiovascular function, is widely used in the treatment of cardiovascular diseases like hypertension, inhibiting the progression of atherosclerosis and reducing mortality in congestive cardiac failure (Aberg and Ferrer 1990, Napoli *et al.* 2004, Repova-Bednarova *et al.* 2013, Hrenak *et al.* 2013, Bencze *et al.* 2013). Because H₂S has been reported to inhibit renin synthesis and release, we studied whether this inhibition is important to the mechanism of H₂S effects on blood pressure. Therefore, we studied the effects of H₂S on blood pressure in the presence and absence of CAP.

S-nitrosoglutathione (GSNO) is the carrier and/or storage form of NO *in vivo* and thus serves as

a reservoir for NO bioactivity (Stamler 1994). Several factors, including the H₂S donor Na₂S, modulate GSNO decomposition resulting in the formation of NO and/or NO-derivatives (Filipovic *et al.* 2012, Ondrias *et al.* 2008, Grman *et al.* 2013, Cortese-Krott *et al.* 2014). These molecules have several biological effects, including smooth muscle relaxation and decrease blood pressure (reviewed in Cacanyiova 2011, Liu *et al.* 2012, Tomaskova *et al.* 2011, Wang 2012, Cortese-Krott *et al.* 2014). In our previous study, we observed that low molecular thiols, such as cysteine, N-acetylcysteine or glutathione, modulate H₂S induced NO release from GSNO (Grman *et al.* 2013). Because CAP also has a thiol moiety and interferes with the NO pathway (Pecháňová 2007, Zandifar *et al.* 2012, Zhang *et al.* 2012), we were interested in determining whether CAP influences H₂S-induced NO release from GSNO and could potentially be involved in H₂S-NO interactions.

Methods

Chemicals

Na₂S was obtained from Alfa Aesar (England). The anesthetics, Narkamon and Rometar, were from Zentiva (Czech Republic), and Zoletil 100 was from Virbac (France). All other chemicals were purchased from Sigma-Aldrich. Na₂S was used as a H₂S donor that dissociates in solution and reacts with H⁺ to yield HS⁻, H₂S and a trace of S²⁻. We use the term H₂S to encompass the total mixture of H₂S, HS⁻ and S²⁻.

MAP, heart and breathing rate measurements

All procedures were approved by the State Veterinary and Food Administration of the Slovak Republic. For the experiments with Na₂S, 240±20 g Wistar rats were used, and for the experiments where Na₂S and CAP were applied, 400±30 g Wistar rats were used. The Wistar rats were anesthetized with Zoletil 100 at a dose of 4.0 mg/100 g body weight (b.w.), and Rometar (Xylazine) was used at a dose of 0.5 mg/100 g b.w. administered intra-muscularly (i.m.). The right jugular vein was prepared and cannulated, and heparin sulphate (25 IU) was administered immediately. The right carotid artery was prepared, cannulated and connected to a Statham P32 Db pressure transducer. After stabilisation of MAP within 15-25 min, Na₂S or CAP dissolved in 100 µl saline was prepared and injected into the jugular vein over a 10 s period. Stock solutions of CAP (20 mmol/l) and Na₂S (100 mmol/l) were prepared only

at the time of measurement and used within a few hours. The recorded analogue signal, low-pass-filter at 1 kHz, was digitalised at 5 kHz (USB-6221, National Instrument, USA) and stored on a PC computer using the DeweSoft 6.6.7 programme for data acquisition and further analysis. Heart rate was evaluated using a fast Fourier transform (FFT) of the digitalised data. From the digitalised data, the frequency of ~70 Hz of FFT was assigned to the breathing rate. The data represent the means ± S.E.M. A paired *t*-test was used to determine the significance of the effect of Na₂S, where P<0.05 was considered statistically significant.

Vasoactivity measurements

In *ex vivo* experiments, the isolated rings of the thoracic aorta were prepared from male Wistar rats and mounted to record isometric tension changes in pneumoxid-oxygenated Krebs-bicarbonate solution as in our previous study (Ondrias *et al.* 2008). The basal tension was set to 1 g before the addition of the drug. The effects of relaxants were given relative to the difference of tension induced by 1 µmol/l noradrenaline and the original tension. This difference was set at 100 %. The precontracted rings were first relaxed by a single addition of GSNO (250 nmol/l) or acetylcholine (30 nmol/l) for 4 min. Then, agents were removed by washing for 20 min. The aorta was precontracted again by 1 µmol/l noradrenaline, Na₂S (40 µmol/l) was applied, and GSNO or acetylcholine were added after 3 min. We also evaluated the involvement of K_{ATP} channel activation (smooth muscle hyperpolarisation) in the effects of Na₂S. The noradrenaline-precontracted rings were first relaxed by the cumulative addition of Na₂S (20, 40, 80, 100 µmol/l). Then, agents were removed by washing for 20 min, the K_{ATP} channel inhibitor glibenclamide (100 µmol/l) was applied 10 min before the repeated addition of noradrenaline and cumulative addition of Na₂S. The vasoactive effects of Na₂S were evaluated as the changes in noradrenaline-induced tone before and after application of glibenclamide (g). The data represent the means ± S.E.M. A paired *t*-test was used to determine the significance of the effect of Na₂S.

CAP and H₂S induced GSNO decomposition

To study the effect of CAP on H₂S-induced NO release from GSNO, the Griess assay and UV-VIS spectrometry were used essentially as described in our previous studies (Ondrias *et al.* 2008, Grman *et al.* 2013). GSNO (100 µmol/l), Na₂S (100 µmol/l) and CAP (100-

800 $\mu\text{mol/l}$) were mixed in buffer (in mmol/l) of 160 KCl, 1 MgCl_2 , 0.1 diethylenetriaminepentaacetic acid (DTPA), 50/25 HEPES/Tris, at pH 7.4 and $23\pm 1^\circ\text{C}$, and incubated for 10 min. The Griess reagent was then added to quantify the NO oxidation product, nitrite (NO_2^-) by absorption (ABS) at 540 nm. To study the effects of CAP on the time dependence of H₂S-induced GSNO decomposition/NO release, CAP (100–400 $\mu\text{mol/l}$) and GSNO (200 $\mu\text{mol/l}$) were included in the buffer (in mmol/l), 100 sodium phosphate, 0.01 DTPA, at pH 7.4, and after adding H₂S (200 $\mu\text{mol/l}$), the kinetics of the GSNO decomposition/NO release were measured using UV-VIS spectrophotometry for 30 min at $23\pm 1^\circ\text{C}$. The NO release from GSNO was signified by a decrease in absorbance at 334 nm and by the formation of an unknown product at 412 nm, as in our previous study.

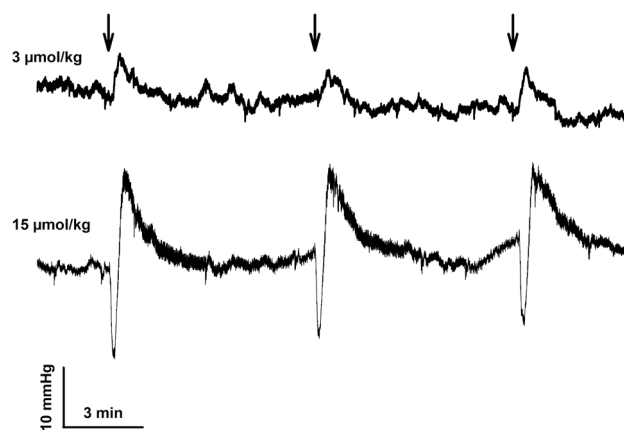


Fig. 1. Representative traces of the time-dependent effect of the repeated i.v. application of Na₂S on MAP of the anesthetized rat. The arrows indicate i.v. application of Na₂S at 3 $\mu\text{mol/kg}$ (upper trace) and 15 $\mu\text{mol/kg}$ (lower trace).

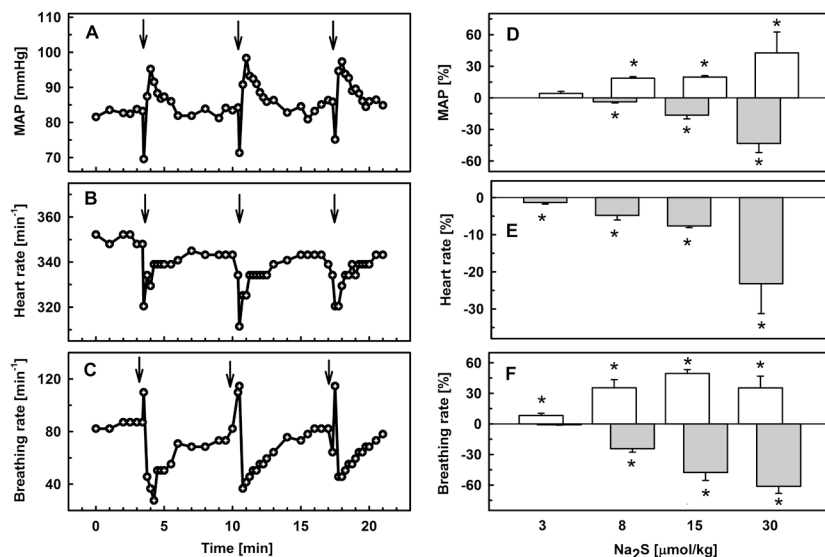


Fig. 2. Time-dependent effect of the repeated i.v. application of 15 $\mu\text{mol/kg}$ Na₂S on the MAP (A), heart (B) and breathing rates (C) of the anesthetized rat. The arrows indicate i.v. application of Na₂S. The concentration-dependent effect of Na₂S on the MAP (D), heart (E) and breathing rates (F) of the anesthetized rat. The control MAP, heart and breathing rates were 82 ± 2 mm Hg, 298 ± 12 min^{-1} , and 77 ± 2 min^{-1} , respectively. Error bars represent the S.E.M. ($n=4-8$; * $p<0.05$ vs. Control).

Results

Na₂S treatment transiently increased breathing rate and decreased heart rate at 3 $\mu\text{mol/kg}$ (Fig. 1). At 8–30 $\mu\text{mol/kg}$, Na₂S biphasically influenced the breathing rate, transiently decreased the heart rate, transiently decreased MAP for ≤ 30 s and increased MAP for ≤ 120 s (Fig. 2).

To study the involvement of the renin-angiotensin system in the effects of Na₂S, we compared the effect of 8 and 16 $\mu\text{mol/kg}$ Na₂S on MAP and heart and breathing rates before, during and after CAP application (2 $\mu\text{mol/kg}$) (Fig. 3). An example of the time-dependent changes observed is shown in Figure 3A,B,C.

Before CAP application, Na₂S exerted a biphasic effect on MAP and breathing rates and transiently decreased heart rate. CAP decreased MAP, but did not significantly influence heart or breathing rates. Na₂S was less effective in decreasing MAP in the presence of CAP, but its effect on the heart and breathing rates did not produce a significant difference from their rates in the absence of CAP (Fig. 2D,E,F). Acetylcholine (0.1 or 1 $\mu\text{mol/kg}$) in the presence of CAP additionally decreased MAP (data not shown). When the effect of CAP wore-off and MAP returned to the control MAP condition, the Na₂S effect was similar to that prior to the CAP application (Fig. 3).

The effects of H₂S on smooth muscle tonus were studied by changes in the vasoactivity of the thoracic

aorta. The application of the NO donor GSNO (250 nmol/l) or the NO-synthase activator acetylcholine (30 nmol/l) induced a vasorelaxant effect in thoracic aorta precontracted with noradrenaline. Acute pretreatment with Na₂S (40 μmol/l) significantly increased the vasorelaxant effect of GSNO; however, it did not affect the vasorelaxation induced by acetylcholine (Fig. 4A). The application of increasing doses of Na₂S on the noradrenaline-precontracted thoracic aorta induced a biphasic effect: the lower concentrations (20, 40 μmol/l)

induced vasoconstriction (Fig. 4B), and the higher concentrations (80, 100 μmol/l) induced vasorelaxation (Fig. 4B). Acute pretreatment with the K_{ATP} channel inhibitor glibenclamide (100 μmol/l) did not affect the vasoconstrictor effect induced by lower Na₂S doses (20 and 40 μmol/l); however, it inhibited the vasorelaxant effect induced by higher doses of Na₂S (8 and 100 μmol/l), and vasoconstriction was observed instead of vasorelaxation (Fig. 4B).

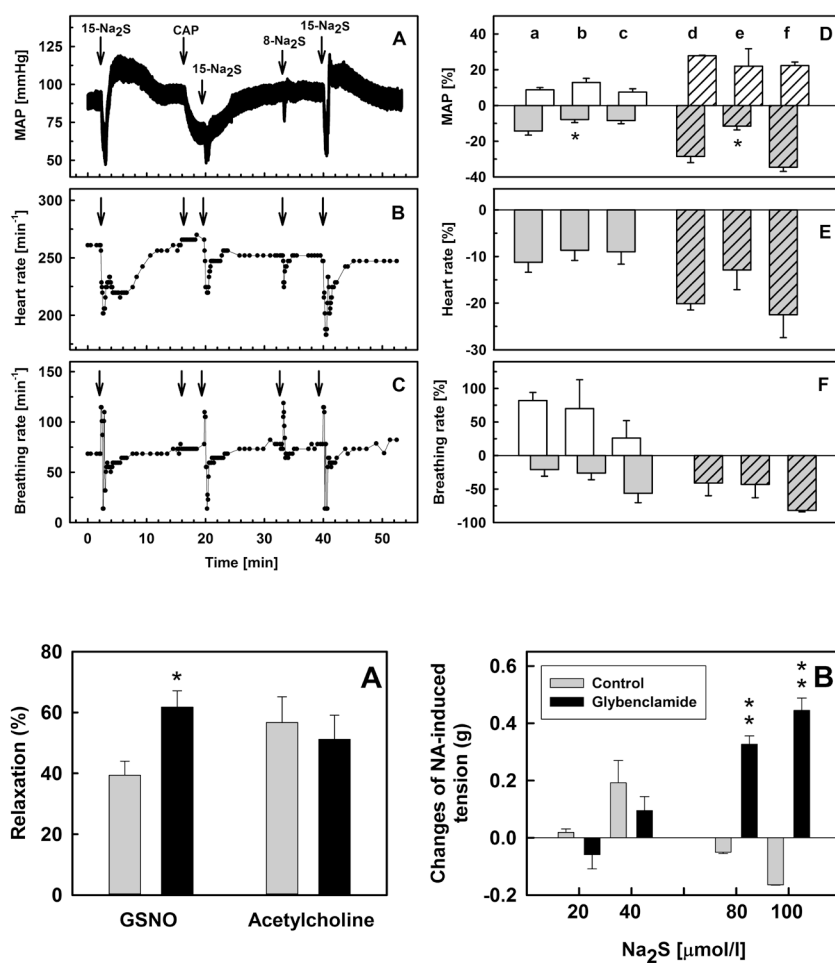


Fig. 3. The time-dependent effects of the sequence of application of Na₂S, CAP, Na₂S in the presence of CAP, and Na₂S on MAP (A, trace), heart (B) and breathing (C) rates. The arrows indicate i.v. application of the drugs. The effects of Na₂S (a, d), Na₂S in the presence of CAP (b, e) and Na₂S after CAP (c, f) on MAP (D), heart (E) and breathing (F) rates. 2 μmol/l CAP; 8 μmol/l Na₂S (open columns); 15 μmol/l Na₂S (hatched columns). The control MAP, heart and breathing rates were 98±3 mm Hg, 271±4 min⁻¹ and 74±7.5 min⁻¹, respectively. The error bars represent the S.E.M. (n=4-8 at 8 μmol/kg; n=3 at 15 μmol/kg; * *p*<0.05 vs. Control).

Fig. 4. The vasoactive effects of GSNO (250 nmol/l) and acetylcholine (30 nmol/l) on noradrenaline (1 μmol/l) precontracted aortic rings before (grey) and after (black) the pretreatment with Na₂S (40 μmol/l) (A). The vasoactive effects of Na₂S on noradrenaline (1 μmol/l) precontracted aortic rings before (grey) and after (black) acute pretreatment with glibenclamide (100 μmol/l) (B). The data are expressed as the mean ± SEM (* *p*<0.05 and ** *p*<0.01 vs. Control).

We studied whether CAP can interfere with the H₂S-induced NO release from GSNO. Two approaches were applied. As detected by the Griess assay, 100 μmol/l Na₂S (but not CAP alone at 100-800 μmol/l) released NO from GSNO (100 μmol/l). However, increased CAP concentrations (100-800 μmol/l) decreased the NO release induced by Na₂S (Fig. 5A). Similar results were obtained using UV-VIS measurement of time dependent Na₂S-induced NO release (ABS at 334 nm). The rate of

Na₂S-induced NO release from GSNO decreased in the presence of increased CAP concentrations (100-400 μmol/l) at pH 7.4 (Fig. 5B). The rate decrease indicates an inhibition of H₂S-induced NO release. The kinetics and amount of unknown product formation detected at 412 nm were also inhibited by CAP (Fig. 5C, pH 7.4). Additionally, the rate of Na₂S-induced NO release was slow at pH 6.0, but CAP (100-400 μmol/l) increased the rate of NO release from GSNO (Fig. 5D).

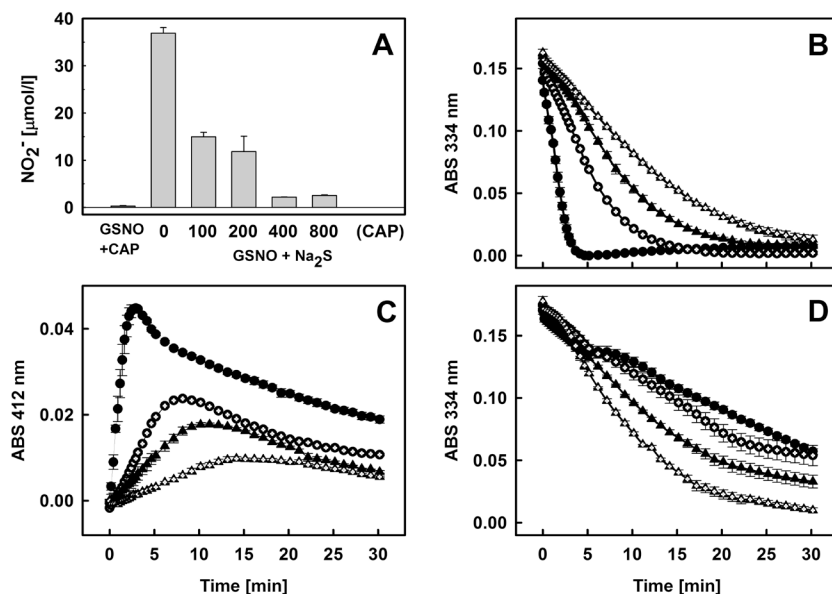


Fig. 5. Concentration-dependent effect of CAP on the release of NO from 100 $\mu\text{mol/l}$ GSNO at pH 7.4 induced by 100 $\mu\text{mol/l}$ Na₂S as indicated by the formation of NO₂⁻ and monitored by the Griess assay (A). The concentration-dependent effect of CAP (100, 200 and 400 $\mu\text{mol/l}$) on the time-dependent effect of Na₂S (200 $\mu\text{mol/l}$) induced GSNO (200 $\mu\text{mol/l}$) decomposition at pH 7.4, as detected with ABS at 334 nm (B) and 412 nm (C). ABS at 334 nm measured at the samples at pH 6.0 (D). Control 200 $\mu\text{mol/l}$ Na₂S + 200 $\mu\text{mol/l}$ GSNO (full circles), and in the presence of CAP: 100 $\mu\text{mol/l}$ (open circles), 200 $\mu\text{mol/l}$ (full triangles) and 400 $\mu\text{mol/l}$ (open triangles).

Discussion

The transient and biphasic effects of H₂S on MAP

In our study, we observed that effects of the H₂S donor Na₂S on blood pressure, heart and breathing rates were transient. Considering that a 250 g rat has a blood volume of approximately 17 ml and a cardiac output of approximately 17 ml in 10 s, the bolus application of 3, 15 and 30 $\mu\text{mol/kg}$ for 10 s delivered 44, 220 and 441 $\mu\text{mol/l}$ Na₂S in the blood, respectively. This treatment is in the range of Na₂S concentrations that release NO from GSNO (Fig. 5). We presume that free H₂S was rapidly eliminated from the rat's blood resulting in transient H₂S effects.

Intravenously injected Na₂S at 8–30 $\mu\text{mol/kg}$ exerted a biphasic transient effect on blood pressure. The K_{ATP} channel has been reported to be a major molecular target of the vasorelaxant and vasodepressor effects of H₂S (Zhao *et al.* 2001). We confirmed this finding using glibenclamide, a K_{ATP} channel inhibitor that significantly inhibited the vasorelaxation induced by higher doses of H₂S (Fig. 4B). Because the transient increase in MAP was observed at lower H₂S concentration than the biphasic effects, we assume that K_{ATP} channels are not involved in the transient MAP increase. Moreover, in *ex vivo* experiments, we showed that the acute pretreatment with glibenclamide did not affect the contractile responses induced by lower doses of H₂S (Fig. 4B).

In our previous studies, we showed that H₂S causes the release of NO from NO donors and that the effects of NO donors on aortic ring relaxation were enhanced in the presence of H₂S donors (Ondrias *et al.*

2008, Bertova *et al.* 2010). In the present study, the H₂S-induced MAP decrease was observed at transient Na₂S blood concentrations, which released NO from GSNO (Fig. 5). Moreover, we confirmed that Na₂S releases NO from the NO donor in *ex vivo* experiments because it significantly augmented the vasorelaxant effect of GSNO (Fig. 4A). However, pretreatment with Na₂S did not affect the vasorelaxant effect of acetylcholine, an NO-synthase activator. Therefore, we assume that H₂S increases NO concentrations *in situ* by releasing NO from endogenous NO donors and not by the activation of the NO-synthase pathway. Thus, the ability of H₂S to decrease MAP may include K_{ATP} channels and NO release from endogenous NO donors.

Biphasic responses of MAP to H₂S could be associated with a sympathetic reflex response, at least the increased phase of the MAP, in accordance with reports for several vasoactive substances such as endothelin, urotensin and apelin (King *et al.* 1990, Gardiner *et al.* 2004, Charles *et al.* 2006). This finding is also in agreement with those of Gines *et al.* (1994), who suggested the sympathetic reflex response issued from the pressure receptor stimulation after detecting aortic hypotension after intravenous acetylcholine injection. The increase of MAP and reduced heart rate observed in our study (Fig. 2) indicate that baroreceptors are also involved in the H₂S effects. Because baroreceptors include channels and H₂S was found to influence membrane channels, we could suppose that H₂S influences baroreceptors through the membrane channels (Chapleau *et al.* 2007, Malekova *et al.* 2009, Peers *et al.* 2012).

The effect of H₂S on heart and breathing rate

We observed that H₂S (3-30 μmol/kg) applied i.v. transiently decreased the heart rate. This finding is supported by the observation of a negative chronotropic effect of H₂S, where the effect of H₂S on membrane channels of pacemaker cells in SA nodes was implicated. In our study, the Na₂S-induced transient decrease in heart rate was accompanied by a decrease and increase in MAP; therefore, we assume that the influence of H₂S on MAP and heart rate results from different H₂S targets and different molecular mechanisms.

We observed that intravenous H₂S influences breathing rate. Our results are supported by the observation that intraperitoneal or peripheral injection of NaSH affected breathing (Kombian *et al.* 1988, Almeida and Guidotti 1999) and that inhalation of H₂S induced a suspended animation-like state in house mice, which included a sharp decrease in the animal's breathing rate, from 120 to 10 breaths/min (Blackstone *et al.* 2005). Because H₂S was found to influence membrane channels involved in membrane excitability and electrical signal transmission (Malekova *et al.* 2009, Peers *et al.* 2012), we assume that the effect of H₂S on these channels is involved in its effect on heart and breathing rates.

Effect of H₂S in the presence of CAP

We observed that H₂S activity on MAP was partially decreased in the presence of CAP. Because acetylcholine additionally decreased MAP in the presence of CAP, we assume that a change in the MAP baseline after CAP addition is not responsible for the weaker H₂S effect. The effect of H₂S on MAP in the presence of CAP can be explained by the reported H₂S-inhibition of renin synthesis and release (Lu *et al.* 2010). However, because the effect of H₂S was reduced but not blocked by CAP, we assume that the renin-angiotensin system was only partially involved. Moreover, the associations among H₂S, the baroreflex mechanism and the autonomic nervous system should be taken into account. Increases in the angiotensin II level in the central nervous system have been shown to affect arterial baroreflex control and increase sympathetic outflow (Gao *et al.* 2005). Because administration of CAP results in the inhibition of angiotensin II synthesis, this compound could mask the possible partial baroreflex-mediated effects of H₂S.

The inhibitory effects of CAP on H₂S-induced NO release from GSNO were similar to other low molecular thiols, such as cysteine, N-acetylcysteine or glutathione, thereby indicating that the thiol group of

CAP is quite important for this effect (Grman *et al.* 2013). If we assume that transient i.v. H₂S concentrations release NO from endogenous NO-donors and decreases MAP, then the inhibition of NO release should lessen the decrease of MAP. Because CAP inhibits the H₂S-induced NO release from GSNO at pH 7.4 *in vitro*, we hypothesize that this effect may also contribute to the lessened MAP decrease. Interestingly, the influence of CAP on H₂S-induced NO release was reversed at pH 6.0 versus pH 7.4. At pH 6.0, CAP increased the NO release from GSNO in the presence of Na₂S. This may indicate a possible pH-dependent influence of CAP on sulfide-nitroso signaling pathways (Tomaskova *et al.* 2011, Grman *et al.* 2013). This idea could be supported by the observation that CAP interferes with the NO pathway (Pecháňová 2007, Zandifar *et al.* 2012, Zhang *et al.* 2012). Its thiol group was found to be important to the prevention of spontaneous hypertension (Pecháňová 2007). In addition to its efficacy as an angiotensin-converting enzyme inhibitor, CAP has several other biological functions. For example, the following have been reported: CAP protects against cystamine-induced duodenal ulcers (Saghaei *et al.* 2012), inhibits cell degeneration and growth (Nagano 2006), scavenges reactive oxygen molecules (Goldschmidt and Tallarida 1991), has renoprotective effects in doxorubicin-induced nephrotoxicity (Hrenák *et al.* 2013), influences mitochondrial ATP production in spontaneously hypertensive rats (Mujkošová *et al.* 2010), and affects potassium and L-type calcium channels (Alvin *et al.* 2011a, b). The diversity of CAP's biological effects have not been fully elucidated. We can assume that some of CAP's numerous biological effects could result from its pH-dependent effects on the H₂S-induced NO release from nitroso-compounds.

Conclusion

The i.v. administration of the H₂S donor Na₂S exerted a transient biphasic effect on MAP, transiently decreased heart rate and influenced breathing rate in anesthetized rats. In addition to activation of K_{ATP} channels, the transient decrease in MAP has been suggested to partially result from the H₂S influence on the renin-angiotensin system and by releasing NO from endogenous NO donors. CAP inhibited the H₂S-induced NO release from GSNO *in vitro*, which could be included in its numerous biological effects. These results

contribute to the understanding of the biological effects of H₂S in the cardiovascular system.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under contract No. APVV-0074-11, by VEGA 2/0050/13, 2/0094/12, 2/0074/14, 2/0067/13 and by Ministry of Health of the Slovak Republic under the project No. 2012/51-SAV-1.

References

- ABERG G, FERRER P: Effects of captopril on atherosclerosis in cynomolgus monkeys. *J Cardiovasc Pharmacol* **15**: S65-S72, 1990.
- ALMEIDA AF, GUIDOTTI TL: Differential sensitivity of lung and brain to sulfide exposure: a peripheral mechanism for apnea. *Toxicol Sci* **50**: 287-293, 1999.
- ALVIN Z, LAURENCE GG, COLEMAN BR, ZHAO A, HAJJ-MOUSSA M, HADDAD GE: Regulation of L-type inward calcium channel activity by captopril and angiotensin II via the phosphatidyl inositol 3-kinase pathway in cardiomyocytes from volume-overload hypertrophied rat hearts. *Can J Physiol Pharmacol* **89**: 206-215, 2011a.
- ALVIN Z, LAURENCE GG, COLEMAN BR, ZHAO A, HAJJ-MOUSSA M, HADDAD GE: Regulation of the instantaneous inward rectifier and the delayed outward rectifier potassium channels by captopril and angiotensin II via the phosphoinositide-3 kinase pathway in volume-overload-induced hypertrophied cardiac myocytes. *Med Sci Monit* **17**: BR165-BR172, 2011b.
- BENCZE M, BEHULIAK M, ZICHA J: The impact of four different classes of anesthetics on the mechanisms of blood pressure regulation in normotensive and spontaneously hypertensive rats. *Physiol Res* **62**: 471-478, 2013.
- BERTOVA A, CACANYIOVA S, KRISTEK F, KRIZANOVA O, ONDRIAS K, TOMASKOVA Z: The hypothesis of the main role of H₂S in coupled sulphide-nitroso signalling pathway. *Gen Physiol Biophys* **29**: 402-410, 2010.
- BLACKSTONE E, MORRISON M, ROTH MB: H₂S induces a suspended animation-like state in mice. *Science* **308**: 518, 2005.
- CACANYIOVA S: Nitric oxide and hydrogen sulfide – contribution of gaseous messengers to the cardiovascular control. *Curr Pharm Biotechnol* **12**: 1292-1293, 2011.
- CHAPLEAU MW, LU Y, ABOUD FM: Mechanosensitive ion channels in blood pressure-sensing baroreceptor neurons. *Curr Top Membr* **59**: 541-567, 2007.
- CHARLES CJ, RADEMAKER MT, RICHARDS AM: Apelin-13 induces a biphasic haemodynamic response and hormonal activation in normal conscious sheep. *J Endocrinol* **189**: 701-710, 2006.
- CORTESE-KROTT MM, FERNANDEZ BO, SANTOS JLT, MERGIA E, GRMAN M, NAGY P, KELM M, BUTLER A, FEELISCH M: Nitrosopersulfide (SSNO⁻) accounts for sustained NO bioactivity of S-nitrosothiols following reaction with sulfide. *Redox Biol* **2**: 234-244, 2014.
- FILIPOVIC MR, MILJKOVIC JLj, NAUSER T, ROYZEN M, KLOS K, SHUBINA T, KOPPENOL WH, LIPPARD SJ, IVANOVIC-BURMAZOVIC I: Chemical characterization of the smallest S-nitrosothiol, HSNO; cellular cross-talk of H₂S and S-nitrosothiols. *J Am Chem Soc* **134**: 12016-12027, 2012.
- GAO L, WANG W, LI YL, SCHULTZ HD, LIU D, CORNISH KG, ZUCKER IH: Sympathoexcitation by central ANG II: Roles for AT1 receptor upregulation and NAD(P)H oxidase in RVLM. *Am J Physiol Heart Circ Physiol* **288**: H2271-H2279, 2005.
- GARDINER SM, MARCH JE, KEMP PA, BENNETT T: Bolus injection of human UII in conscious rats evokes a biphasic haemodynamic response. *Br J Pharmacol* **143**: 422-430, 2004.
- GINES FF, VICENTE KE, RICCA R: Right ventricle systolic pressure increase following intravenous acetylcholine injection. *Acta Physiol Pharmacol Ther Latinoam* **44**: 77-84, 1994.
- GOLDSCHMIDT JE, TALLARIDA RJ: Pharmacological evidence that captopril possesses an endothelium-mediated component of vasodilation: Effect of sulfhydryl groups on endothelium-derived relaxing factor. *J Pharmacol Exp Ther* **257**: 1136-1145, 1991.

- GRMAN M, MISAK A, JACOB C, TOMASKOVA Z, BERTOVA A, BURKHOLZ T, DOCOLOMANSKY P, HABALA L, ONDRIAS K: Low molecular thiols, pH and O₂ modulate H₂S-induced S-nitrosoglutathione decomposition – ¹⁴NO release. *Gen Physiol Biophys* **32**: 429-441, 2013.
- HRENAK J, ARENDAŠOVA K, RAJKOVICOVA R, AZIRIOVA S, REPOVA K, KRAJCIROVICOVA K, CELEC P, KAMODYOVA N, BARTA A, ADAMCOVA M, PAULIS L, SIMKO F: Protective effect of captopril, olmesartan, melatonin and compound 21 on doxorubicin-induced nephrotoxicity in rats. *Physiol Res* **62** (Suppl 1): S181-S189, 2013.
- KING AJ, PFEFFER JM, PFEFFER MA, BRENNER BM: Systemic hemodynamic effects of endothelin in rats. *Am J Physiol* **258**: H787-H792, 1990.
- KOMBIAN SB, WARENYCIA MW, MELE FG, REIFFENSTEIN RJ: Effects of acute intoxication with hydrogen sulfide on central amino acid transmitter systems. *Neurotoxicology* **9**: 587-595, 1988.
- LIU YH, LU M, HU LF, WONG PTH, WEBB GD, BIAN JS: Hydrogen sulfide in the mammalian cardiovascular system. *Antioxid Redox Signal* **17**: 141-185, 2012.
- LU M, LIU YH, GOH HS, WANG JJX, YONG QC, WANG R, BIAN JS: Hydrogen sulfide inhibits plasma renin activity. *J Am Soc Nephrol* **21**: 993-1002, 2010.
- MALEKOVA L, KRIZANOVA O, ONDRIAS K: H₂S and HS⁻ donor NaHS inhibits intracellular chloride channels. *Gen Physiol Biophys* **28**: 190-194, 2009.
- MUJKOSOVA J, ULICNA O, WACZULIKOVA I, VLKOVICOVA J, VANCOVA O, FERKO M, POLAK S, ZIEGELHOFFER A: Mitochondrial function in heart and kidney of spontaneously hypertensive rats: influence of captopril treatment. *Gen Physiol Biophys* **29**: 203-207, 2010.
- NAGANO M: Inhibitory effect of captopril on cell degeneration and growth. *Exp Clin Cardiol* **11**: 195-197, 2006.
- NAPOLI C, SICA V, DE NIGRIS F, PIGNALOSA O, CONDORELLI M, IGNARRO LJ, LIGUORI A: Sulfhydryl angiotensin-converting enzyme inhibition induces sustained reduction of systemic oxidative stress and improves the nitric oxide pathway in patients with essential hypertension. *Am Heart J* **148**: e5, 2004.
- ONDRIAS K, STASKO A, CACANYIOVA S, SULOVA Z, KRIZANOVA O, KRISTEK F, MALEKOVA L, KNEZL V, BREIER A: H₂S and HS⁻ donor NaHS releases nitric oxide from nitrosothiols, metal nitrosyl complex, brain homogenate and murine L1210 leukaemia cells. *Pflugers Arch* **457**: 271-279, 2008.
- PECHÁŇNOVÁ O: Contribution of captopril thiol group to the prevention of spontaneous hypertension. *Physiol Res* **56** (Suppl 2): S41-S48, 2007.
- PEERS C, BAUER CC, BOYLE JP, SCRAGG JL, DALLAS ML: Modulation of ion channels by hydrogen sulfide. *Antioxid Redox Signal* **17**: 95-105, 2012.
- PREDMORE BL, LEFER DJ: Development of hydrogen sulfide-based therapeutics for cardiovascular disease. *J Cardiovasc Transl Res* **3**: 487-498, 2010.
- REPOVA-BEDNAROVA K, AZIRIOVA S, HRENAK J, KRAJCIROVICOVA K, ADAMCOVÁ M, PAULIS L, ŠIMKO F: Effect of captopril and melatonin on fibrotic rebuilding of the aorta in 24 hour light-induced hypertension. *Physiol Res* **62** (Suppl 1): S135-S141, 2013.
- SAGHAEI F, KARIMI I, JOUYBAN A, SAMINI M: Effects of captopril on the cysteamine-induced duodenal ulcer in the rat. *Exp Toxicol Pathol* **64**: 373-377, 2012.
- STAMLER JS: Redox signaling: nitrosylation and related target interactions of nitric oxide. *Cell* **78**: 931-936, 1994.
- TOMASKOVA Z, BERTOVA A, ONDRIAS K: On the involvement of H₂S in nitroso signaling and other mechanisms of H₂S action. *Curr Pharm Biotechnol* **12**: 1394-1405, 2011.
- WANG R: Physiological implications of hydrogen sulfide: a whiff exploration that blossomed. *Physiol Rev* **92**: 791-896, 2012.
- ZANDIFAR E, SOHRABI BEHESHTI S, ZANDIFAR A, HAGHJOOY JAVANMARD S: The effect of captopril on impaired wound healing in experimental diabetes. *Int J Endocrinol* **2012**: ID 785247, 2012.
- ZHANG MZ, WANG S, YANG S, YANG H, FAN X, TAKAHASHI T, HARRIS RC: Role of blood pressure and the renin-angiotensin system in development of diabetic nephropathy (DN) in eNOS^{-/-} db/db mice. *Am J Physiol* **302**: F433-F438, 2012.
- ZHAO W, ZHANG J, LU Y, WANG R: The vasorelaxant effect of H₂S as a novel endogenous gaseous KATP channel opener. *EMBO J* **20**: 6008-6016, 2001.