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Effects of Hydrogen Sulphide on the Isolated Perfused Rat Heart

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تأثيرات غاز كبريتيد الهيدروجين على قلب جرذي مروي ومعزول

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الملخص: **الأهداف**: تم تعريف غاز كبريتيد الهيدروجين على أنه غاز متميز الجزيء في الجسم، إذ تم الكشف مسبقا على احتوائه على خصائص تساعد على رخاوة الأوعية. تهدف الدراسة إلى الكشف عن تأثيرات غاز هايدروسلفايد الصوديوم (الذي يعطي بدوره كبريتيد الهيدروجين) على معدل نبضات القلب والضغط المترتب عليه في البطين الأيسر والتدفق في الشريان التاجي في قلب الفأر المعزول المروي. **الطريقة**: تم استخدام طرق تحضير القلب المعزول (لاندجيندروف) للبحث عن تأثير جرعة محددة لغاز كبريتيد الهيدروجين أثناء استخدام مثبطات النشاط الكيميائي أو عدم استخدامه في معدل نبضات القلب والضغط في البطين الأيسر والتدفق في الشريان التاجي. **النتائج**: تسببت هيدروسولفايد الصوديوم بانخفاض كبير في معدل ضربات القلب عند شدة تركيز($3 - 10$) مول ($P < 0.05$) هذا الانخفاض تثبط بشكل جزئي بواسطة جليبينكلامايد – المانع لقناة KATP ($P < 0.05$)، المثبط لتصنيع أكسيد النتريك ($P < 0.001$)، والمثبط أيضا لإنتاج المثيلين الأزرق ($P < 0.001$). بينما ثبط ال ($H-89$) زيادة تدفق الشريان التاجي المتسبب عن هايدروسلفايد الصوديوم ($P < 0.001$). من جهة أخرى، قلل هايدروسلفايد الصوديوم (أيا كان تركيزه) الضغط في البطين الأيسر بشكل معتد إحصائيا ($P < 0.001$). وفي وجود الجليبينكلامايد و ($H-89$) كان الوقت المطلوب لتخفيض الضغط في البطين الأيسر بسبب هايدروسلفايد الصوديوم أطول ($P < 0.001$) بينما سبب المثيلين الأزرق و($L-NAME$) نقصا للاستجابة لهايدروسلفايد الصوديوم ($P < 0.05$، $P < 0.01$) بالتتابع. **الخلاصة**: أدى استخدام غاز كبريتيد الهيدروجين إلى خفض معدل نبضات القلب ومعدل الضغط في البطين الأيسر وزيادة التدفق في الشريان التاجي في قلب الفأر المعزول ولكن آليات العمل لم تقم بتوضيح النتائج بشكل تام.

مفتاح الكلمات: كبريتيد الهيدروجين ؛ القلب ؛ مرسل الغاز لاندجيندروف ؛ ارتخاء وعائي

ABSTRACT: ***Objectives:*** Hydrogen sulphide has been identified as a gas signalling molecule in the body, and has previously been shown to have vasorelaxant properties. The aim of the study was to investigate the effects of sodium hydrosulphide (NaHS), a hydrogen sulphide donor, on heart rate (HR), left ventricular developed pressure (LVDP) and coronary flow (CF) in the isolated perfused rat heart. ***Methods:*** A Langendorff isolated heart preparation was used to investigate the effect of a dose range of sodium hydrosulphide, in the presence and absence of inhibitors, on heart rate, left ventricular developed pressure and coronary flow. ***Results:*** Sodium hydrosulphide caused a significant decrease in heart rate at a concentration of 10-3 M (P <0.001). This decrease was partially inhibited by glibenclamide, a K_{ATP} channel blocker (P <0.05); L-NAME, a nitric oxide synthase inhibitor (P <0.001), and methylene blue (P <0.001), but not by H-89, a protein kinase A inhibitor. Sodium hydrosulphide significantly increased coronary flow at concentrations of 10-4 – 10-3M (P <0.05). This response was significantly increased in the presence of L-NAME (P <0.001) and methylene blue (P <0.001), whereas H-89 inhibited the increase in coronary flow due to sodium hydrosulphide (P <0.001). Sodium hydrosulphide significantly decreased LVDP at all concentrations (P <0.001). In the presence of glibenclamide and H-89, the time period of the decrease in LVDP due to sodium hydrosulphide was extended (P <0.001), whereas methylene blue and L-NAME caused a significant reduction in the response to sodium hydrosulphide (P <0.05, P <0.01 respectively). ***Conclusion:*** Sodium hydrosulphide reduced heart rate and LVDP, and increased coronary flow in the isolated perfused rat heart; however, the mechanisms of action could not be fully elucidated.

Keywords: Hydrogen sulphide; Heart; Langendorff; H_2S gasotransmitter; Vasorelaxation

Advances in Knowledge

1. *This study identifies a sodium hydrosulfide dependant decrease in heart rate and left ventricular pressure and an increase in coronary flow.*
2. *This study also identifies the potential cell signalling pathways via which these physiological changes take place.*

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Application to Patient Care

1. *Hydrogen sulphide has been identified as a gaseous signalling molecule alongside nitric oxide and carbon monoxide mediating a range of physiological processes.*
2. *Hydrogen sulphide has been shown to abolish the effects seen during myocardial ischaemia reperfusion injury and has the potential of being translated into a clinical setting.*

It is now well established that hydrogen sulphide (H_2S) gas has effects on the heart; however, to date, the cardiovascular effects of both endogenous and exogenous H_2S have not been fully elucidated. Zhao et al. reported that an intravenous bolus injection of H_2S at 2.8 and 14 μmol/Kg body weight caused a significant transient decrease in blood pressure in anaesthetised rats, which was partially antagonised by glibenclamide (a potassium adenosine triphosphate [K_{ATP}] ion channel blocker).[1] The heart rate was not significantly affected by H_2S injection. An early study on sulphydryl reagents on the isolated perfused guinea-pig heart reported an increase in coronary flow and heart rate.[2] *Geng et al.* reported that sodium hydrosulphide (NaHS), a H_2S donor, inhibited left ventricular developed pressure in the isolated perfused rat heart in a concentration-dependent manner over a range of 10-6–10-3 M.[3] However, they found a decrease in heart rate and coronary flow only at a concentration of 10-3 M NaHS which was partially inhibited by glibenclamide.[3] *Sun et al.* found that NaHS impeded contraction of isolated rat cardiomyocytes by inhibiting L-type calcium channels.[4] No effects on K_{ATP} channel currents or on levels of cAMP (cyclic adenosine monophosphate) or cGMP (cyclic guanosine monophosphate) were found. H_2S was found to have a negative chronotropic action on pacemaker cells in the sinoatrial node of rabbit heart, possibly via the opening of K_{ATP} channels.[5]

A number of recent studies have shown that H_2S is cardioprotective in myocardial ischaemia and ischaemia-reperfusion injury.[6-12] Some of these studies found evidence that the cardioprotective mechanism is mediated via the opening of K_{ATP} channels;[7,10,11,13,14] however, it is controversial whether mitochondrial K_{ATP} channels are involved.[11,13,15] Inhibition of nitric oxide production attenuated the cardioprotective effects of NaHS, suggesting some synergy between nitric oxide (NO) and H_2S.[13] There is some evidence that the protein kinase C pathway,[15,16] upregulation of cyclooxygenase-2[17]2[17] or upregulation of heat shock protein 72[12] could be involved in cardioprotection.

H_2S is produced endogenously from the amino acid L-cysteine by two enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE). CBS has been shown to have no activity or expression in human cardiovascular related tissues.[18,19] Conversely, CSE expression and endogenous production of H_2S have been shown in the rat portal vein, thoracic aorta[20] and heart.[3,6] The expression of CSE has been identified in vascular smooth muscle cells, but not in the endothelium, whereas CBS expression was not detected in vascular tissue.[1]

There appears to be some similarities between H_2S, NO and carbon monoxide (CO) in terms of their effects, and mechanisms of action and interactions between them have been reported.[21]Hosoki *et al.* were the first to suggest synergy between H_2S and NO in relaxing vascular smooth muscle.[19] They demonstrated a left-ward shift in the dose-response curve for relaxation of rat thoracic aorta by NaHS in the presence of two different NO donors, sodium nitroprusside and morpholinosydnonimine. They reported that a low concentration of H_2S enhanced the smooth muscle relaxant effect of NO by up to 13-fold. However, Zhao and Wang found that low doses of NaHS shifted the dose-response relaxation curve for sodium nitroprusside to the right in rat aortic rings, suggesting that H_2S inhibited the vasorelaxant effect of NO.[22]

The primary aim of this study was to investigate the effects of a concentration range of NaHS, a H_2S donor, on heart rate, left ventricular developed pressure and coronary flow in the isolated perfused rat heart using the Langendorff model.

Methods

Male Sprague-Dawley male rats (250–300g body weight) were used in all the experiments. All animals were from the same source, fed a standard diet and housed in the same conditions. All animals received humane care in accordance with the UK Guidance on the Operation of the Animals (Scientific Procedures) Act 1986. Animal protocols

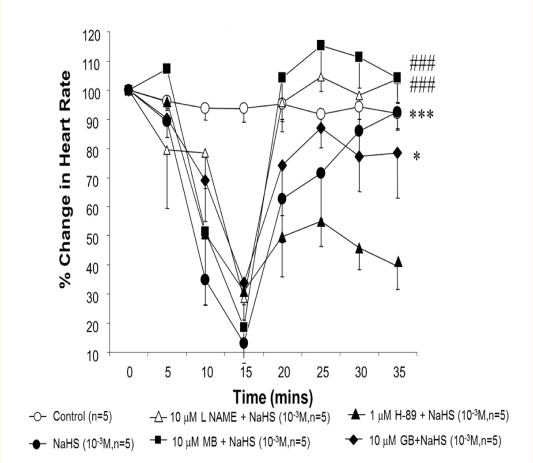

Figure 1a: Percent change in heart rate over time due to infusion of Sodium hydrosulphide (NaHS) (10-3 M) in the presence of glibenclamide (10 μM), H89 (1 μM), methylene blue (10 μM) and L-nitro-arginine-methyl ester (L-NAME) (10 μM) (mean ± Standard error of the mean (SEM), n = 5). The initial heart rate in each case was taken as 100%. Hearts were infused with the inhibitors for 15 minutes, followed by NaHS for 15 minutes and then recovery was monitored for a further 20 minutes.

*Note: ***P <0.001 vs. Control; * P <0.05 vs. NaHS; ### P <0.001 vs. NaHS.*

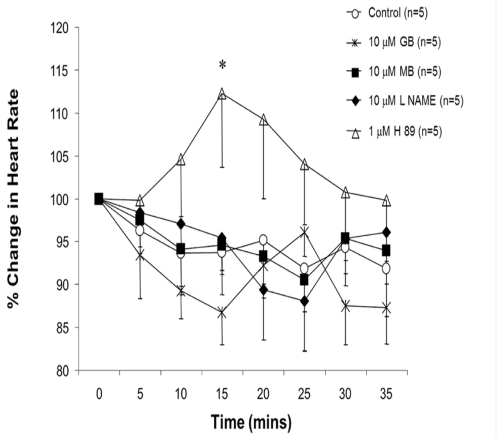

Figure 1b: Percent change in heart rate over time due to infusion of glibenclamide (10 μM), H89 (1 μM), methylene blue (10 μM) and L-nitro-arginine-methyl ester (L-NAME) (10 μM) (mean ± Standard error of the mean (SEM), n = 5). The initial heart rate in each case was taken as 100%. Hearts were infused with the inhibitors for 35 minutes. H89 alone caused a significant increase in heart rate compared to control (P <0.02).

*Note: * P <0.05 vs. Control.*

were approved by the Committee of Animal Care and Supply of Coventry University, UK.

All chemicals were obtained from Sigma (Poole, UK). The Krebs Heinseleit buffer contained (mM) NaCl 118.5, $NaHCO_3$ 25, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$, 1.7, and glucose 12, methylene blue (a guanylate cyclase inhibitor), glibenclamide and H-89 (a protein kinase A inhibitor) were dissolved in dimethyl sulphoxide (DMSO) initially and then diluted with a KH buffer. L-nitro-arginine-methyl ester (L-NAME) was dissolved in distilled water. NaHS (a H_2S donor) was dissolved in a KH buffer. The rate of infusion of agents was 1% of coronary flow to achieve the final concentrations indicated.

The animals were killed by cervical dislocation followed by exsanguination. Hearts were rapidly excised and placed in ice-cold Krebs-Heinseleit (KH) buffer solution. The aortic stump was rapidly mounted onto a cannula attached to a standard Langendorff set up. Hearts were perfused with KH buffer, gassed with 95% O_2 5% CO_2 (pH 7.4) and maintained at 37.5°C. The temperature was constantly monitored by a thermocouple inserted into the right ventricle.

A latex balloon was positioned in the left ventricle via an insertion performed in the left atrial appendage and inflated to 5–10 mmHg. The balloon was attached to a pressure transducer connected to a Harvard amplifier to determine the left ventricular developed pressure (LVDP). Heart rate was monitored via the electrocardiogram (ECG) recorded using electrodes connected to a Harvard isolated preamplifier. Hearts exhibiting arrhythmia were discarded. Readings were taken at 5 min intervals using a Thermo array recorder WR7700 (Western Graphtec, USA). Coronary flow was measured at 5 mins intervals by collecting the perfusate draining from the perfused heart over a fixed time period.

Hearts were allowed to stabilise for 20 mins following mounting on the Langendorff set up. Hearts were randomly assigned to the following protocols:

a) Control group (n = 5): hearts were infused using a Harvard infusion/withdrawal pump with KH buffer at room temperature without the addition of any agents for 15 minutes at an infusion rate of 0.13 ml/min and allowed to recover for a further 20 mins; b) Concentration-effect group: hearts were infused with a range of concentrations of NaHS (M) of 10^{-5} (n = 6), 10^{-4} (n = 5) and $3x10^{-3}$ (n = 5) for a period of 15 minutes, followed by a further 20 minutes of recovery; c) Hearts were allowed to stabilise for 20 mins with KH buffer perfusion and were then

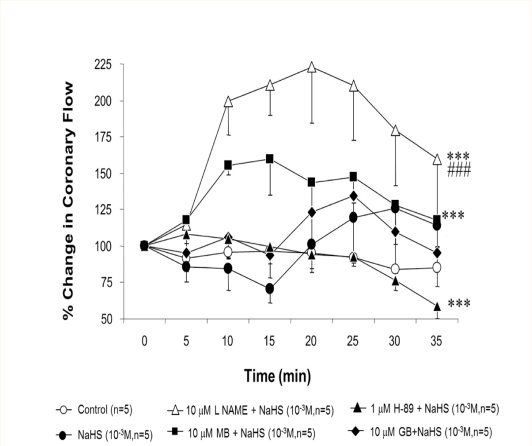

Figure 2a: Percent change in coronary flow over time due to infusion of Sodium hydrosulphide (NaHS) (10-3 M) in the presence of glibenclamide (10 μM), H89 (1 μM), methylene blue (10 μM) and L-nitro-arginine-methyl ester (L-NAME) (10 μM) (mean ± Standard error of the mean (SEM), n = 5). The initial coronary flow was taken as 100% in each case. Hearts were infused with the inhibitors for 15 minutes, followed by NaHS for 15 minutes and then recovery was monitored for a further 20 minutes.

*Note: *** P <0.001 vs. NaHS; ### P <0.001 vs. Control.*

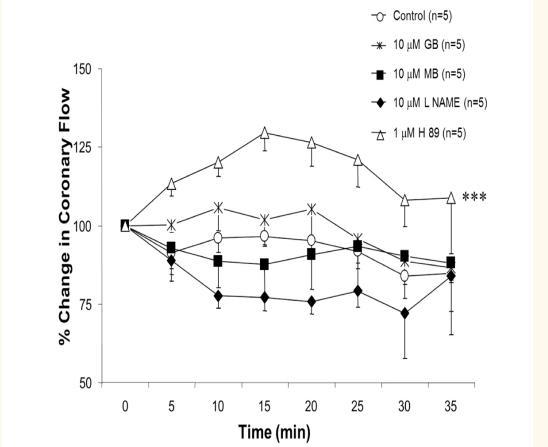

Figure 2b: Percent change in coronary flow above over time due to infusion of glibenclamide, H89, methylene blue and L-nitro-arginine-methyl ester (L-NAME) (mean ± Standard error of the mean (SEM), n = 5). The initial coronary flow in each case was taken as 100%. Hearts were infused with the inhibitors for 35 minutes. H-89 alone caused a significant increase in coronary artery flow (P <0.001).

*Note: *** P <0.001 vs. Control.*

exposed with either 10 μM glibenclamide (n = 5), or 10 μM MB (a guanylate cyclase inhibitor) (n = 5), or 10 μM L-NAME (n = 5), or 1 μM H-89 (n = 5) for 35 mins; d) Hearts were allowed to stabilise for 20 mins with KH buffer and were then exposed to either 10 μM glibenclamide (n = 5), 10 μM MB (n = 5), 10 μM L-NAME (n = 5) or 1 μM H-89 (n = 5) for 15 minutes. Hearts were then exposed to 10^{-3} M NaHS for 15 mins followed by a further 20 mins for recovery.

Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS, Version 10.5). Data were compared using analysis of covariance (ANCOVA) or one-way analysis of variance (ANOVA) with Fisher's protected least mean squares difference post hoc test. Differences were considered significant where P <0.05. All data are expressed as mean ± standard error of the mean (SEM).

Results

Results are presented as the percentage mean of the stabilisation period. NaHS caused a significant decrease in heart rate compared with control at a concentration of 10^{-3} M (P <0.001) [Figure 1a]. This decrease was partially, but significantly, inhibited by glibenclamide (P <0.05), by L-NAME (P <0.001), and by methylene blue (P <0.001), but not by H-89 [Figure 1a]. Glibenclamide, L-NAME and methylene blue alone had no significant effect on heart rate, but H-89 alone caused a significant increase in heart rate compared to control subjects (P = 0.05) [Figure 1b].

NaHS caused a significant increase in coronary flow compared to control at a concentration of 10^{-3} M (P <0.05) [Figure 2a]. Glibenclamide did not block the response to NaHS [Figure 2a]. H-89 alone caused a significant increase in coronary flow (P <0.001) [Figure 2b], but it significantly inhibited the increase in coronary flow due to NaHS (P <0.001) [Figure 2a]. Methylene blue alone had no significant effect on coronary flow, but it significantly augmented the increase due to NaHS (P <0.001) [Figure 2a]. L-NAME alone had no significant effect on coronary flow [Figure 2b], however L-NAME with NaHS caused a profound, highly significant increase in coronary flow compared to control (P <0.001) and compared to NaHS alone (P <0.001) [Figure 2b].

NaHS significantly decreased LVDP at all concentrations (P <0.001) compared with control (data not shown). Glibenclamide did not affect the initial response to NaHS, but significantly inhibited the recovery in LVDP following NaHS treatment (P

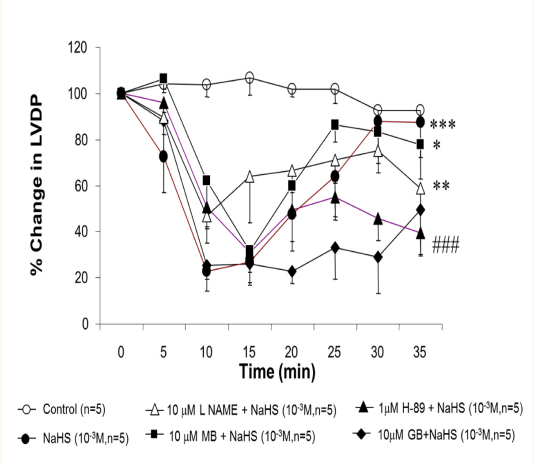

Figure 3a: Percent change in Left ventricular Developed Pressure (LVDP) over time due to different concentrations of Sodium hydrosulphide (NaHS) (10-3 M) in the presence of glibenclamide, H89, methylene blue and L-nitro-arginine-methyl ester (L-NAME) (mean ± Standard error of the mean (SEM), n = 5). The initial LVDP was taken as 100% in each case. Hearts were infused with the inhibitors for 15 minutes, followed by NaHS for 15 minutes and then recovery was monitored for a further 20 minutes.

*Note: *** P <0.001 vs. Control; ### P <0.001 vs. NaHS; * P <0.05 vs. NaHS; ** P <0.01 vs. NaHS.*

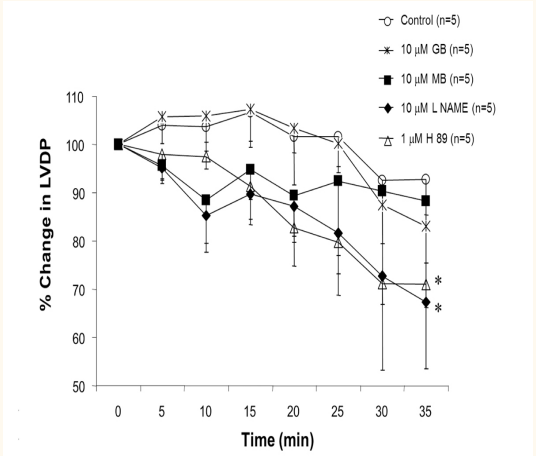

Figure 3b: Percent change in Left ventricular Developed Pressure (LVDP) over time due to infusion of glibenclamide, H89, methylene blue and L-nitro-arginine-methyl ester (L-NAME) (mean ± Standard error of the mean (SEM), n = 5). The initial LVDP in each case was taken as 100%. Hearts were infused with the inhibitors for 35 minutes.

*Note: * P <0.03 vs. Control.*

<0.001) (Figure 3a). H-89 alone caused a significant decrease in LVDP over time (P = 0.03) [Figure 3b], and H-89 significantly decreased the recovery in LVDP after NaHS treatment (P <0.001) [Figure 3a]. Methylene blue caused a significant reduction in the response to NaHS (P <0.05) [Figure 3a]. L-NAME alone caused a significant reduction in LVDP over time (P = 0.03) [Figure 3b], but it significantly reduced the effect of NaHS on LVDP (P = 0.01) [Figure 3a].

Discussion

The H_2S donor NaHS caused significant reductions in heart rate and LVDP, but increased coronary flow. The effect on LVDP concurs with the known role of H_2S as a muscle relaxant,[1,20,22,23] and suggests that it has a similar effect on cardiac muscle. The effects of NaHS on LVDP reported here support the findings of Geng *et al.*[3] In a study on isolated rat cardiomyocytes, Sun *et al.* reported that NaHS reduced contraction and inhibited L-type calcium channels.[4] The vasodilatory effect of H_2S in increasing coronary flow agrees with reported effects on peripheral blood vessels *in vitro.*[1,20,22] However, Geng *et al.* reported a decrease in coronary flow in a similar model.[3]

The negative chronotropic effect on heart rate, which agrees with the findings of Geng *et al.*[3] must presumably be due to H_2S influencing ion channels and ion currents in the pacemaker cells. Xu *et al.*[5] have reported that NaHS decreases the rate of pacemaker firing in the sinoatrial node in rabbit. In previous reports of the effects of H_2S on the heart in vivo, Zhao *et al.*[1] and Geng *et al.*[3] reported that an intravenous bolus injection of H_2S or NaHS caused a significant transient decrease in blood pressure, but the heart rate was not significantly affected. The heart may well respond differently *in vivo* compared to *in vitro*. There are probably differences in dose between these experiments and the present study. A crude estimate of the maximum dose range the heart was exposed to in the *in vivo* experiments of Zhao *et al.* is 4.5 x 10^{-5} – 2.4 x 10^{-4} M, assuming a rat body weight of 375 g and a blood volume of 22 ml.[1] Geng *et al.* used a dose of 2.8 µmol/Kg body weight.[3]

In the present study, the effect of H_2S on heart rate seems to partially involve K_{ATP} channels, as it was inhibited by glibenclamide. This agrees with the findings of Xu *et al.* who suggested that H_2S influences pace maker cell depolarisation by opening K_{ATP} channels.[5] H_2S, acting by opening K_{ATP} channels, would hyperpolarise the membranes of pacemaker cells, thus reducing their rate of depolarisation and decreasing heart rate. The lack

of effect of H-89 suggests that H_2S is not exerting its negative chronotropic effect via the Cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway. The significant increase in heart rate due to H-89 alone might be due to the fact that H-89 is not very specific in its action of inhibiting PKA. Alternatively, the normal control of heart rate could involve the (cAMP/PKA) pathway. The effect of L-NAME and methylene blue in inhibiting the action of H_2S in decreasing heart rate suggests that the mechanism involves the stimulation of production of NO and the guanylate cyclic guanosine monophosphate (cGMP) pathway. In comparison, Kojda *et al*. found that L-arginine had a positive chronotropic effect in rat heart, whereas methylene blue reduced heart rate.[29]

H_2S has been previously shown to have vasodilatory effects in the rat heart[1,20,22] and, in the present study, H_2S increased coronary flow in isolated perfused hearts indicating dilation of coronary blood vessels. Conversely, Geng *et al.* reported a decrease in coronary flow by NaHS in a similar model.[3] Blockade of K_{ATP} channels with glibenclamide, in the present study, did not inhibit the vasodilatory effect of H_2S, suggesting that the mechanism of action of H_2S was not via opening K_{ATP} channels. This is in contrast to the findings of Zhao *et al*. using peripheral blood vessels.[1] H-89 alone significantly increased coronary flow which could be due to its other actions apart from inhibiting PKA. However, H-89 inhibited the vasodilatory effect of NaHS, suggesting that the mechanism of action of H_2S in this case is via the cAMP/PKA pathway. This finding agrees with that of Kimura who showed that physiological concentrations of H_2S increased the production of cyclic AMP in neurones and oocytes in culture.[27]

Methylene blue and L-NAME both caused a highly significant increase in the vasodilatory effect of NaHS, which shows that inhibition of nitric oxide synthesis and signalling via guanylate cyclase augmented the effect of H_2S. These findings were reproducible and were highly statistically significant. This suggests that endogenous production of NO inhibits the vasodilatory effect of H_2S in coronary blood vessels; however, it is well known that NO is a vasodilator of coronary blood vessels. Zhao *et al.* showed that L-NAME, an inhibitor of endogenous NO production, significantly shifted the H_2S dose-response relaxation curve to the right, decreasing the potency of H_2S, in rat aortic rings, and similar effects were obtained by removing the endothelium from the aortic rings.[1] They also showed that the addition of a specific inhibitor of soluble guanylate cyclase, 1 H-[1,2,4]oxadiazolo[4,3,-*a*]quinoxalin-1-one, significantly increased the relaxant effect of H_2S; however, synergy between NO and H_2S has also been reported. Hosoki *et al.,* demonstrated a left-ward shift in the dose-response curve for relaxation of rat thoracic aorta by NaHS in the presence of two different NO donors, sodium nitroprusside and morpholinosydnonimine.[15] Cheung and Schulz reported that glutathione and glutathione disulphide caused coronary vasodilation which was mediated by a NO and guanylate cyclase-dependent mechanism.[26] It is possible, in the current study, that endogenously produced NO could stimulate the production of a factor which opposes the actions of H_2S.

Both H-89 and L-NAME caused a significant reduction in LVDP. The reduction in LVDP by H-89 could be due to effects other than on PKA. The depressor effect of L-NAME would suggest that the maintenance of LVDP requires the production of NO, although NO is generally known to be a muscle relaxant. It has been previously reported that a NO donor SPM3672 increased maximal left ventricular pressure in the isolated rat heart and increased levels of cGMP in rat cardiomyocytes.[28] The positive inotropic effects of NO donors in rat heart have been confirmed by the same group and others.[25,26] H-89 extended the time period of the decrease in LVDP following NaHS treatment, suggesting that normal recovery involved the cAMP/PKA pathway. L-NAME and methylene blue significantly inhibited the response to NaHS, suggesting that the mechanism of relaxation of cardiac muscle by H_2S involves NO production and guanylate cyclase. As H_2S binds to heme groups, similarly to NO, it is possible that guanylate cyclase is a target. Glibenclamide did not inhibit the initial depression of LVDP by NaHS, suggesting that the mechanism of action did not involve the opening of K_{ATP} channels. Indeed, glibenclamide extended the time period of the depression of LVDP due to NaHS and thus inhibited recovery of the heart from NaHS treatment. This could be interpreted as an involvement of the opening of K_{ATP} channels in the recovery from H_2S treatment. However, it is unclear how the opening of K_{ATP} channels and the resultant hyperpolarisation could increase LVDP.

Conclusion

NaHS, an H_2S donor, significantly reduced heart rate and LVDP, and increased coronary flow in the isolated perfused rat heart; however, the mechanisms of action could not be fully elucidated.

CONFLICT OF INTEREST

The authors reported no conflict of interest.

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References

1. Zhao W, Zhang J, Li Y, Wang R. The vasorelaxant effects of H2S as a novel endogenous KATP channel opener. EMBO J 2001; 20:6008–16.
2. Gailis L, Nguyen MH. The effect of sulfhydryl reagents on the heart rate and coronary flow of the isolated perfused guinea-pig heart. Arch Int Pharmacodyn Ther 1975; 218:19–28.
3. Geng B, Yang J, Qi Y, Zhao J, Pang Y, Du J, Tang C. H2S generated by heart in rat and its effects on cardiac function. Biochem Biophys Res Comm 2004; 313:362–8.
4. Sun YG, Cao YX, Wang WW, Ma SF, Yao T, Zhu YC. Hydrogen sulphide is an inhibitor of L-type calcium channels and mechanical contraction in rat cardiomyocytes. Cardiovasc Res 2008; 79:632–41.
5. Xu M, Wu YM, Li Q, Wang X, He RR. Electrophysiological effects of hydrogen sulfide on pacemaker cells in the sinoatrial nodes of rabbits. Sheng Li Xue Bao 2008; 60:175–80.
6. Zhu YZ, Wang ZJ, Ho P, Loke YY, Zhu YC, Huang SH, et al. Hydrogen sulfide and its possible roles in myocardial ischemia in experimental rats. J Appl Physiol 2007; 102:261–8.
7. Johansen D, Ytrehus K, Baxter GF. Exogenous hydrogen sulfide (H2S) protects against regional myocardial ischemia-reperfusion injury – evidence for a role of KATP channels. Basic Res Cardiol 2006; 101:53–60.
8. Bian JS, Yong QC, Pan TT, Feng ZN, Ali MY, Zhou S, et al. Role of hydrogen sulfide in the cardioprotection caused by ischemic preconditioning in the rat heart and cardiac myocytes. J Pharmacol Exp Ther 2006; 316:670–8.
9. Sivarajah A, McDonald MC, Thiermermann C. The production of hydrogen sulfide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in the rat. Shock 2006; 26:154–61.
10. Rossoni G, Sparatore A, Tazzari V, Manfredi B, Del Soldato P, Berti F. The hydrogen sulphide-releasing derivative of diclofenac protects against ischaemia-reperfusion injury in the isolated rabbit heart. Br J Pharmacol 2008; 153:100–9.
11. Ji Y, Pang QF, Xu G, Wang L, Wang JK, Zeng YM. Exogenous hydrogen sulfide postconditioning protects isolated rat hearts against ischemia-reperfusion injury. Eur J Pharmacol 2008; 587:1–7.
12. Bliksøen M, Kaljusto ML, Vaage J, Stensløkken KO. Effects of hydrogen sulphide on ischaemia-reperfusion injury and ischaemic preconditioning in the isolated, perfused rat heart. Eur J Cardiothorac Surg 2008; 34:344–9.
13. Pan TT, Feng ZN, Lee SW, Moore PK, Bian JS. Endogenous hydrogen sulfide contributes to the cardioprotection by metabolic inhibition preconditioning in the rat ventricular myocytes. J Mol Cell Cardiol 2006; 40:119–30.
14. Zhang Z, Huang H, Liu P, Tang C, Wang J. Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening KATP channels. Can J Physiol Pharmacol 2007; 85:1248–53.
15. Pan TT, Neo KL, Hu LF, Yong QC, Bian JS. H2S preconditioning-induced PKC activation regulates intracellular calcium-handling in rat cardiomyocytes. Am J Physiol Cell Physiol 2008; 294:169–77.
16. Hu LF, Pan TT, Neo KL, Yong QC, Bian JS. Cyclooxygenase-2 mediates the delayed cardioprotection induced by hydrogen sulfide preconditioning in isolated rat cardiomyocytes. Pflugers Arch 2008; 455:971–8.
17. Chen P, Poddar R, Tipa E, Dibello PO, Moravec CD, Robinson K, et al. Homocysteine metabolism in cardiovascular cells and tissues: implications for hyperhomocysteinemia and cardiovascular disease. Adv Enzyme Regul 1999; 39:93–109.
18. Bao L, Vleck C, Paces V, Kraus JP. Identification and tissue distribution of human cystathionine beta-synthase mRNA isoforms. Arch Biochem Biophys 1998; 350:95–103.
19. Hosoki R, Matsiki N, Kimura H. The possible role of hydrogen sulphide as an endogenous smooth muscle relaxant in synergy with nitric oxide. Biochem Biophysic Res Commun 1997; 237:527–31.
20. Warenycia MW, Goodwin LR, Benishin CG, Reiffenstein RJ, Francom DM, Taylor JD, et al. Acute hydrogen sulphide poisoning demonstration of selective uptake of sulfide by brainstem by measurement of brain sulfide levels. Biochem Pharmacol 1989; 38:973–81.
21. Goodwin LR, Francom D, Dieken FP, Taylor JD, Warenycia MW, Reiffenstein RJ, et al. Determination of sulphide in brain tissue by gas dialysis/ ion chromatography: post mortem studies and two case reports. J Analyte Toxicol 1989; 13:105–9.

22. Carson RJ, Seyffarth G, Mian R, Maddock H. Interactions between Gasotransmitters. In: Wang R. Ed. Signal Transduction and the Gasotransmitters: NO, CO and H2S in Biology and Medicine. Totowa: Humana Press, 2004. Pp. 33–55.

23. Zhao W, Wang R. H2S-induced vasorelaxation and underlying cellular and molecular mechanisms. Am J Physiol Heart Circ Physiol 2002; 283:474–80.

24. Teague B, Asiedu S, Moore PK. The smooth muscle relaxant effect of hydrogen sulfide in vitro: Evidence for a physiological role to control intestinal contractility. Brit J Pharmacol 2002; 137:139–45.

25. Sidhu R, Singh M, Samir G, Carson R.J. L-Cysteine and sodium hydrogen sulphide inhibit spontaneous contractility in isolated pregnant rat uterine strips in vitro. Pharmacol Toxicol 2001; 88:198–203.

26. Beauchamp RO, Bus JS, Popp J.A, Boreiko CJ, Andjelkovich D A. A critical review of the literature on hydrogen sulfide toxicity. Crit Rev Toxicol 1984; 13:25–97.

27. Kimura H. Hydrogen sulfide induces cyclic AMP and modulates the NMDA receptor. Biochem Biophys Res Comm 2000; 267:129–33.

28. Kojda G, Brixius K, Kottenberg K, Nix P, Schluter KD, Piper HM, et al. The new NO donor SPM3672 increases cGMP and improves contraction in rat cardiomyocytes and isolated heart. Eur J Pharmacol 1995; 284:315–9.

29. Kojda G, Kottenberg K,, Stasch JP, Schror K, Noack E. Positive inotropic effect of exogenous and endogenous NO in hypertrophic rat hearts. Br J Pharmacol 1997; 122:813–20.