Analysis of erectile responses to H2S donors in the anesthetized rat

Ryan C. Jupiter,1 Daniel Yoo,1 Edward A. Pankey,1 Vishwaradh V. G. Reddy,1 Justin A. Edward,1 David J. Polhemus,2 Taylor C. Peak,1 Prasad Katakam,1 and Philip J. Kadowitz1

1Department of Pharmacology, Tulane University School of Medicine, New Orleans, Louisiana; and 2Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, Louisiana

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Jupiter RC, Yoo D, Pankey EA, Reddy VG, Edward JA, Polhemus DJ, Peak TC, Katakam PV, Kadowitz PJ. Analysis of erectile responses to H2S donors in the anesthetized rat. Am J Physiol Heart Circ Physiol 309: H835–H843, 2015. First published June 26, 2015; doi:10.1152/ajpheart.00293.2015.—Hydrogen sulfide (H2S) is a biologically active endogenous gasotransmitter formed in penile tissue that has been shown to relax isolated cavernosal smooth muscle. In the present study, erectile responses to the H2S donors sodium sulfide (Na2S) and sodium hydrosulfide (NaHS) were investigated in the anesthetized rat. Intracavernosal injections of Na2S in doses of 0.03–1 mg/kg increased intracavernosal pressure and transiently decreased mean arterial pressure in a dose-dependent manner. Blood pressure responses to Na2S were rapid in onset and short in duration. Responses to Na2S and NaHS were similar at doses up to 0.3 mg/kg, after which a plateau in the erectile response to NaHS was reached. Increases in intracavernosal pressure in response to Na2S were attenuated by tetraethylammonium (K+ channel inhibitor) and iberiotoxin (large-conductance Ca2+-activated K+ channel inhibitor), whereas glibenclamide [ATP-sensitive K+ (KATP) channel inhibitor] and inhibitors of nitric oxide (NO) synthase, cyclooxygenase, and cytochrome P-450 epoxygenase had no effect. These data indicate that erectile responses to Na2S are mediated by a tetraethylammonium- and iberiotoxin-sensitive mechanism and that KATP channels, NO, or arachidonic acid metabolites are not involved. Na2S did not alter erectile responses to sodium nitroprusside (NO donor) or cavernosal nerve stimulation, indicating that neither NO nor cGMP metabolism are altered. Thus, Na2S has erectile activity mediated by large-conductance Ca2+-activated K+ channels. It is suggested that strategies that increase H2S formation in penile tissue may be useful in the treatment of erectile dysfunction when NO bioavailability, KATP channel function, or poor responses to PGE1 are present.

hydrogen sulfide; hydrogen sulfide donors; erectile function; tetraethylammonium; iberiotoxin

NEW & NOTEWORTHY

The present study shows that H2S donors have erectile activity in the rat. The erectile response is mediated by large-conductance Ca2+-activated K+ channels and are independent of nitric oxide, arachidonic acid metabolites, and ATP-sensitive K+ channels. These data suggest that H2S donors may be useful in the treatment of erectile dysfunction.

HYDROGEN SULFIDE (H2S) is the third gasotransmitter and may play a role in the regulation of erectile function (8, 11, 26, 35, 36). H2S is synthesized from L-cysteine by at least four enzymes, with cystathionine γ-lyase and cystathionine β-synthase being the main enzymes in penile tissue (8, 11, 14, 23, 38–40, 42). It has been reported that H2S or H2S donors relax corpora cavernosal smooth muscle and that intracavernosal injections of sodium hydrosulfide (NaHS; H2S donor) increased penile length and pressure in the primate and increased intracavernosal pressure (ICP) in the rat (8, 11, 26, 35, 36). The mechanism by which H2S relaxes corpora cavernosal smooth muscle is uncertain (12, 19, 33, 45–47). It has been hypothesized that the KATP channel may be important in mediating the erectile response to H2S (32). It has also been suggested that the proerectile effect of H2S may involve an inhibitory effect on cGMP breakdown or metabolism (1–3, 32, 33, 45). The present study was undertaken to investigate erectile responses to the H2S donors sodium sulide (Na2S) and NaHS in the anesthetized rat and to determine the role of NO, KATP channels, BKCa channels, arachidonic acid metabolites and cytochrome P-450 epoxygenase pathway, and cGMP breakdown in mediating erectile responses to H2S donors. The results of these experiments show that the H2S donors Na2S and NaHS have significant erectile activity, with high doses of Na2S producing large increases in ICP. The results indicate that erectile responses to Na2S are mediated by a tetraethylammonium (TEA)- and iberiotoxin-sensitive mechanism and indicate that NO, KATP channels, arachidonic acid metabolites, and cGMP breakdown or metabolism are not involved. It is suggested that erectile responses to the H2S donor are mediated in large part by the activation of BKCa channels in the corpora cavernosa of the rat.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of the Tulane University School of Medicine approved the experimental protocol used in this study, and all procedures were conducted in accordance with institutional guidelines. For these experiments, adult male Sprague-Dawley rats were anesthetized with a 100 mg/kg ip injection of Inactin (thiobutabarbital, Sigma-Aldrich, St. Louis, MO). Supplemental doses of Inactin were given intraperitoneally as needed to maintain a uniform level of anesthesia. Body temperature was maintained with a heating lamp. The trachea was cannulated with a short segment of polyethylene (PE)-240 tubing to maintain a patent airway, and animals spontaneously breathed room air enriched with a low flow stream of 100% O2, which we have observed to improve respiratory function. The left carotid artery was catheterized with PE-50 tubing for the measurement of systemic arterial pressure. For the catheterization of the corpora cavernosum, a midline incision was performed along the scrotum. The bulbocavernosal and ischiocavernosal muscles were exposed. The ischiocavernosal muscle was separated from the corpora cavernosum and then divided to expose a portion of the cavernosal tissue, and this procedure was performed.
bilaterally. ICP was measured with a 25-gauge needle inserted into the left crura entering the sinusoidal tissue of the corpora cavernosa. The needle was connected to PE-50 tubing filled with heparinized saline and attached to a pressure transducer. Systemic arterial pressure and ICP were measured with Namic Perceptor DT pressure transducers and a data-acquisition system (Biopac MP 100A-CE, Santa Barbara, CA). ICP and mean arterial pressure (MAP) obtained by electronic averaging of the pressure signals were continuously recorded, displayed, and stored on a Dell PC. The left jugular vein was catheterized with PE-50 tubing for the systemic administration of drugs and fluids. A 26-gauge needle was placed in the right crura of the penis for the administration of Na2S, NaHS, cromakalim (KATP channel activator), TEA (K+ channel inhibitor), iberiotoxin (BKCa channel inhibitor), Nω-nitro-arginine methyl ester [l-NAME; NO synthase (NOS) inhibitor], sodium meclofenamate (cyclooxygenase inhibitor), miconazole (cytochrome P-450 epoxygenase inhibitor), sodium nitroprusside (SNP; NO donor), and propargylglycine (H2S inhibitor), miconazole (cytochrome P-450 epoxygenase inhibitor), sodium nitroprusside (SNP; NO donor), and propargylglycine (H2S synthesis inhibitor). Maximal ICP in response to intracavernosal injection of the vasodilator agents was measured at the peak of the increase in ICP. The area under the curve (AUC) of the increase in ICP was measured to characterize the total erectile response (in mmHg/s). The AUC takes into account the increase in ICP and duration of the increase in ICP and provides a measure of the total response to an erectile stimulus that can be used for comparative purposes.

For cavernosal nerve stimulation experiments, the bladder and prostate were exposed through a midline abdominal incision, and the major pelvic ganglia and cavernosal nerve were identified posterolateral to the prostate on one side. A stainless steel stimulating electrode (Harvard Apparatus, Holliston, MA) was placed around the nerve, and the cavernosal nerve was stimulated at 5–8 V with 5-ms pulses at 4, 8, and 16 Hz for 60 s using a SD9 square-wave stimulator (Grass Medical Instruments, Quincy, MA) was placed around the nerve, and a rest period of 10 min was allowed between cavernosal nerve stimulation trials.

Medical Instruments, Quincy, MA). A rest period of 10 min was allowed between cavernosal nerve stimulation trials.

Drugs. l-NAME, Na2S, NaHS, cromakalim, sodium meclofenamate, TEA, iberiotoxin, propargylglycine, and SNP (Sigma-Aldrich) were dissolved in 0.9% NaCl, and solutions were made on a daily basis for H2S donors and 2–3 times/week for other agents. Glybenclamide (Sigma-Aldrich) was dissolved in 10% cremophor, 10% transcutol, and 80% saline solution. Miconazole (Sigma-Aldrich) was dissolved in DMSO. Glybenclamide (Sigma-Aldrich) was prepared in 4 ml propylene glycol and sonicated. Concentrated NaOH (300 µg), 100% ethanol (1.7 ml), and Tris (4 ml, pH 8.4) were added to the resulting solution. For all intracavernosal injections, drug solutions were given at a fixed volume of 50 µl and injected through a 26-gauge needle into the right crura. ICP was allowed to return to the baseline value before subsequent injections. This period varied from ~15–30 min depending on the dose and agent injected. Drug solutions were prepared on a daily basis for H2S donors and 2–3 times/week for other agents.

Statistics. Data are expressed as means ± SE and were analyzed using one-way ANOVA and a Student’s t-test for paired and unpaired data. A P value of <0.05 was used as the criterion for statistical significance.

RESULTS

Erectile responses to Na2S. Erectile responses to the H2S donors Na2S and NaHS were investigated in the anesthetized rat, and these data are shown in Figs. 1, 2, and 6. Intracavernosal injection of Na2S in doses of 0.03–1 mg/kg produced dose-related increases in ICP, ICP/MAP, and AUC, which reflects the total erectile response and response duration, and dose-dependent decreases in MAP (Fig. 1). The time course of the increase in ICP and decrease in MAP in response to intracavernosal injection of Na2S (0.5 mg/kg) is shown in Fig. 2. The onset and recovery of the decrease in MAP in response to intracavernosal injection of Na2S were rapid. The rapid decrease in MAP in response to intracavernosal injection of the H2S donor is caused by the rapid systemic absorption of this diffusible molecule. The increase in ICP was slower in onset than the decrease in MAP in response to Na2S, and the duration of the ICP increase was much longer than the duration of the decrease in MAP (Fig. 2). The increase in ICP occurred at the same time MAP was returning to the baseline value, and the maximum increase in ICP in response to intracavernosal

Fig. 1. Bar graphs showing increases in intracavernosal pressure (ICP), ICP/mean arterial pressure (ICP/MAP), area under the curve (AUC), and response duration and decreases in MAP in response to intracavernosal injections of Na2S in doses of 0.03–1 mg/kg. n = 9–12. *P < 0.05 (by ANOVA).
injection of Na2S occurred when MAP had returned to the baseline value (Fig. 2).

Mechanisms of erectile responses to Na2S. The role of NO, KATP channels, cyclooxygenase and cytochrome P-450 epoxygenase pathway products, and BKCa channels in mediating erectile responses to Na2S was investigated, and these data are shown in Fig. 3. The increases in ICP in response to intracavernosal injections of Na2S were not attenuated after the intravenous injections of l-NAME (50 mg/kg), glybenclamide (10 mg/kg), sodium meclofenamate (5 mg/kg), or miconazole (50 mg/kg) in separate groups of animals (Fig. 3). The increases in ICP in response to intracavernosal injections of Na2S were significantly attenuated after administration of TEA at a dose of 30 mg/kg ic (Fig. 3). A record from an experiment showing the effect of TEA on the increase in ICP in response to intracavernosal injection of Na2S is shown in Fig. 2.

The effect of iberiotoxin on the erectile response to Na2S is shown in Fig. 4. After administration of iberiotoxin at a dose of 100 μg/kg ic, the increases in ICP, ICP/MAP, and AUC in response to intracavernosal injection of Na2S (300 μg/kg) were significantly decreased, whereas the erectile response to intracavernosal injection of SNP (1 μg/kg) was not altered (Fig. 4).

Effect of Na2S on responses to SNP and cavernosal nerve stimulation. It has been proposed that inhibition of cGMP breakdown or metabolism by an effect on phosphodiesterase 5 may play a role in mediating or modulating the erectile response to H2S (32). The effect of the H2S donor Na2S on the response to the NO donor SNP and to cavernosal nerve stimulation, which is mediated in large part by the release of NO, the activation of soluble guanylate cyclase, and increased
corpora cavernosal levels of cGMP, was investigated, and these data are shown in Fig. 5. The increases in ICP in response to intracavernosal injections of SNP (0.3 μg/kg) or to cavernosal nerve stimulation at 8 Hz were not altered by intracavernosal injections of Na2S (0.3 mg/kg; Fig. 5). In some experiments, a second or third intracavernosal injection of Na2S (0.3–0.5 mg/kg) was given, and increases in ICP in response to intracavernosal injections of SNP or to cavernosal nerve stimulation were not altered.

Erectile responses to NaHS. Erectile responses to the H2S donor NaHS were also investigated, and these data are shown in Fig. 6. Intracavernosal injections of NaHS in doses of 0.03–0.3 mg/kg produced dose-related increases in ICP, ICP/MAP, AUC, and duration and dose-related decreases in MAP
Fig. 5. A: bar graphs showing the effect of Na$_2$S (0.3 mg/kg ic) on the decrease in MAP and increase in ICP, ICP/MAP, and AUC in response to intracavernosal injection of the NO donor SNP (0.3 μg/kg ic). B: bar graphs showing the effect of Na$_2$S on the increase in ICP, ICP/MAP, and AUC in response to cavernosal nerve stimulation at 8 Hz, 5 V, for 60 s. The erectile response to the NO donor SNP or to cavernosal nerve stimulation, which releases endogenous NO, were not altered. n = 6–7 per group. Paired comparison is shown.
The dose-response curve for the increases in ICP and ICP/MAP reached a plateau, and increases in ICP were not much greater at the higher doses of NaHS (Fig. 6). The increases in ICP in response to NaHS were significantly attenuated by treatment with TEA at a dose of 30 mg/kg ic (Fig. 7).

Intravenous administration of L-NAME significantly increased MAP and had small inconsistent effects on ICP. Intravenous administration of glybenclamide, sodium meclofenamate, and miconazole had small inconsistent effects on MAP and ICP. Intracavernosal injection of TEA or iberiotoxin had inconsistent effects on baseline ICP and MAP.

**Effect of propargylglycine on the response to cavernosal nerve stimulation.** The effect of the H2S synthesis inhibitor propargylglycine on the response to electrical stimulation of the cavernosal nerve was investigated, and these data are shown in Fig. 8. The increase in ICP in response to cavernosal nerve stimulation was enhanced significantly at 2, 4, and 8 Hz after the administration of propargylglycine at a dose of 50 mg/kg iv (Fig. 8).

**DISCUSSION**

The results of the present study show that the H2S donors Na2S and NaHS have significant erectile activity in the anesthetized rat. New findings in this study indicate that erectile responses to the H2S donor Na2S are mediated by a TEA- and iberiotoxin-sensitive mechanism and are not altered by inhibitors of NOS, KATP channels, and cyclooxygenase or cytochrome P-450 epoxygenase pathways. The present study shows that intracavernosal injections of H2S donors produce dose-related increases in ICP, ICP/MAP, and AUC. The increase in ICP was slower in onset and longer in duration than the fall in MAP so that driving pressure (MAP) had returned to the control or baseline value when the increase in ICP was reaching a maximum. The explanation for the slower onset and longer duration of the increase in ICP is uncertain but may be related to delivery and the mechanism of action and of inactivation of the H2S donor in the corpora cavernosa. The decrease in MAP in response to intracavernosal injection of H2S donors is due to systemic absorption and is observed with intracavernosal injection of many proerectile agents (21, 22, 29). The present data are consistent with results in the literature indicating that H2S donors have erectile activity in vivo (13, 14, 34, 48). The present findings extend previous observations by providing additional data on the kinetics of the erectile response to H2S donors and mechanistic data on the erectile response to H2S donors in the intact rat. The mechanism by which H2S increases erectile activity is uncertain (32). It has been suggested that an effect on the NO-cGMP pathway or K_ATP channels may be involved in mediating erectile responses to H2S (6, 8, 10–12, 17, 18, 24, 26–28, 34, 35, 43, 46). It has also been suggested that H2S may act by inhibiting cGMP breakdown in a manner similar to sildenafil (32–34).

In the present study, the role of NO and effects on cGMP breakdown or metabolism were investigated. The effect of L-NAME at a dose that was shown to inhibit the erectile response to cavernosal nerve stimulation had no significant effect on the increase in ICP in response to intracavernosal injections of Na2S, indicating that NOS and NO were not involved in mediating the response in the rat (9, 21). In addition, erectile responses to the NO donor SNP were not altered by intracavernosal injections of Na2S, indicating that responses are independent of an effect on NO-cGMP signaling. This observation along with the results showing that increases in ICP in response to cavernosal nerve stimulation, which are mediated in large part by NO release and an increase in cGMP levels, are not enhanced indicate that the H2S donors are not acting to inhibit phosphodiesterase 5, which would result in an enhanced response to exogenous, nerve, and endothelium-released NO.

H2S generated from NaHS has been reported to relax isolated vascular and corpora cavernosal smooth muscle, and H2S has been reported to decrease arterial pressure in the rat (8, 11,
activation in mediating erectile responses to H\textsubscript{2}S donors in the rat.

The role of arachidonic acid metabolites of cyclooxygenase and cytochrome P-450 epoxygenase pathways in mediating erectile response to Na\textsubscript{2}S was investigated by administration of sodium meclofenamate or miconazole in doses reported to inhibit the cyclooxygenase and cytochrome P-450 epoxygenase pathways, respectively (5, 9, 30, 31). These data suggest that metabolites in cyclooxygenase or epoxygenase pathways are not involved in mediating or modulating erectile responses to the H\textsubscript{2}S donor in the rat.

It has recently been reported that relaxant responses to Na\textsubscript{HS} in vas deferens and tracheal smooth muscle and small mesenteric arteries are mediated by activation of BK\textsubscript{Ca} channels and are blocked by TEA (15, 16, 25, 44). In the present study, the

26, 34, 35). It has been reported that vasorelaxant responses are mediated by a mechanism involving K\textsubscript{ATP} channels in endothelial and vascular smooth muscle cells (6, 10, 17, 24, 27, 28, 43). It has also been proposed that H\textsubscript{2}S is a major endothelium-derived factor that causes hyperpolarization and vasorelaxation by activating K\textsubscript{ATP} channels (6, 10, 17, 24, 27, 28, 43). In the present study, the increase in ICP and decrease in MAP in response to intracavernosal injections of Na\textsubscript{2}S were not attenuated by the administration of the K\textsubscript{ATP} channel antagonist glybenclamide in a dose that inhibited responses to the K\textsubscript{ATP} channel agonist cromakalim (data not shown). These results do not provide evidence in support of a role for K\textsubscript{ATP} channel
effects of TEA and iberiotoxin on erectile responses to Na₂S were investigated, and the results show that increases in ICP in response to intracavernosal injections of Na₂S are attenuated by TEA and iberiotoxin in doses that did not alter erectile responses to the NO donor SNP. These data suggest that the increase in ICP in response to Na₂S is mediated by the activation of BKCa channels and is consistent with previous studies showing that H₂S dilates rat mesenteric arteries by BKCa channels and that intermittent hypoxia in rats increases myogenic tone by the loss of H₂S activation of these channels (15, 16).

The results of electrophysiological experiments show that the resting membrane potential of smooth muscle cells in small penile arteries and the corpora cavernosa is approximately −40 mV and that BKCa channels are expressed on smooth muscle and endothelial cells in penile arteries and the corpora cavernosa (4, 20). It has been reported that BKCa channels play an important role in regulating erectile function and that channel knockout produces erectile dysfunction in mice (41). Endogenous H₂S insufficiency has been associated with low testosterone levels and erectile dysfunction (37). It would be important to measure the effect of H₂S donors on membrane potential and Ca²⁺ movements in imaging studies in smooth muscle and endothelial cells in the corpora cavernosa and small penile arteries.

H₂S is synthesized in penile tissue and relaxes isolated corpora cavernosal smooth muscle strips (8, 11, 14, 26). H₂S donors have been observed to promote penile erection in rats and primates (8, 13, 14, 33, 35, 36). However, the characteristics and mechanism of the erectile response to H₂S are uncertain. The present results show that the H₂S donors Na₂S and NaHS have significant erectile activity in the rat. These results show that the erectile and hypotensive responses have a different time course and that the maximum increase in ICP occurs when MAP had returned to the baseline value. The different time course of the changes in ICP and MAP may be related to delivery and the mechanism of action and of inactivation of H₂S in the corpora cavernosa and systemic vascular bed. The increase in ICP in response to the H₂S donor Na₂S is attenuated by TEA, whereas l-NAME, glybenclamide, sodium meclofenamate, and miconazole had no significant effect, and the erectile response to Na₂S is attenuated by iberiotoxin. These data suggest that the erectile response to the H₂S donor is mediated by a TEA- and iberiotoxin-sensitive mechanism that may involve BKCa channels and that NO, Kᵦᵦᵦ channels, and cyclooxygenase or cytochrome P-450 epoxygenase pathway metabolites have no major role. The observation that erectile responses to H₂S donors are not attenuated by l-NAME, an agent that inhibits the synthesis of NO, the principle mediator of erectile function, suggests that they are not dependent on an intact innervation. These results indicate that erectile responses in the rat and H₂S-induced relaxation of vas deferens and rat mesenteric arteries are mediated by a similar TEA-sensitive mechanism (12, 16, 25).

The effect of propargylglycine, an inhibitor of H₂S synthesis, on the response to cavernosal nerve stimulation was also investigated. In these experiments, inhibition of H₂S synthesis enhanced the erectile response to cavernosal nerve stimulation. It has been reported that the response to transmural nerve stimulation was enhanced by inhibition of H₂S synthesis in isolated human corpora cavernosal strips (8). However, in another study (35), responses to transmural nerve stimulation were decreased by inhibition of H₂S formation. In addition, a study (36) in the rat showed that the response to cavernosal nerve stimulation was reduced at 20 Hz. These results suggest that endogenous H₂S may play a role in modulating neurogenic erectile responses; however, the difference in results in different studies indicates that further investigation is required to determine the role of endogenous H₂S formation on erectile function. The results of the present study may be interpreted to suggest that a H₂S-based therapeutic strategy may, however, be useful in the treatment of erectile dysfunction in patients in which NO formation or bioavailability, KATP channel function, or responses to PGE₁ are severely impaired and testosterone levels are reduced.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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