Hydrogen sulfide as a mediator of human corpus cavernosum smooth-muscle relaxation

Roberta d’Emmanuele di Villa Bianca, Raffaella Sorrentino, Pasquale Maffia, Vincenzo Mirone, Ciro Imbimbo, Ferdinando Fusco, Raffaele De Palma, Louis J. Ignarro, and Giuseppe Cirino

Department of Experimental Pharmacology, University of Naples Federico II, Via Domenico Montesano 49, 80131 Naples, Italy; Interdepartmental Research Center for Sexual Medicine, University of Naples Federico II, Via Paisans 5, 80131 Naples, Italy; Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow G40 NR, Scotland; Department of Experimental Medicine, Second University of Naples, 80100 Naples, Italy; and Department of Molecular and Medical Pharmacology, University of California School of Medicine, Los Angeles, CA 90095

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Hydrogen sulfide (H2S), like nitric oxide (NO), was best known as a toxic pollutant until recent years when it has been proposed to be a gaseous neurotransmitter. In the recent literature, H2S is becoming recognized as a mediator of physiological and/or pathological processes (1–3). H2S is present in micromolar concentrations in blood (4), and it can be synthesized from L-cysteine (L-Cys), which acts as a substrate for the synthesis of H2S. Human penile tissue possesses both CBS and CSE, and tissue homogenates efficiently convert L-Cys to H2S. CBS and CSE are localized in the muscular trabeculae and the smooth-muscle component of the penile artery, whereas CSE but not CBS is also expressed in peripheral nerves. Exogenous H2S [sodium hydrogen sulfide (NaHS)] or L-Cys causes a concentration-dependent relaxation of strips of human corpus cavernosum. L-Cys relaxation is inhibited by the CBS inhibitor, aminooxyacetic acid (AOAA). Electrical field stimulation of human penile tissue, under resting conditions, causes an increase in tension that is significantly potentiated by either propargylglycin (PAG; CSE inhibitor) or AOAA. In rats, NaHS and L-Cys promote penile erection, and the response to L-Cys is blocked by PAG. Our data demonstrate that the L-Cys/H2S pathway mediates human corpus cavernosum smooth-muscle relaxation.

Results

Real-Time Quantitative RT-PCR and Western Blot Studies. Quantitative RT-PCR demonstrated the presence of both CBS and CSE mRNA in HCC tissue (Fig. 1A). CBS and CSE were also expressed as proteins as demonstrated by Western blot studies (Fig. 1B).

H2S Production in HCC. HCC generated detectable amounts of H2S (Fig. 1C). The biosynthesis of H2S was increased by 3-fold over basal values after incubation of tissue homogenates with L-Cys, the CBS/CSE substrate (Fig. 1C). PAG (10 mM), AOAA (1 mM), or the combination of both inhibitors significantly inhibited the increase in H2S production stimulated with L-Cys. Therefore, HCC is capable of synthesizing H2S from L-Cys.

Immunohistochemistry of CBS and CSE. The immunohistochemistry study shows that HCC tissue expressed a robust signal for CSE and to a lesser extent for CBS (Fig. 2). Bundles of muscular tissue in trabeculae showed a clear immunoreactivity for CSE (Fig. 2A and B) and CBS (Fig. 2D). In addition, immunoreactivity for CBS was also present in the vascular smooth-muscle cells of penile arteries (Fig. 2C). When we attempted to perform an immunohistochemistry study on peripheral nerves to visualize CBS and CSE, we found a negative staining (Fig. 2G and H). Because nerve morphology was particularly affected by the antigen retrieval in paraffin sections, we performed the immu-

In the present work, by using human corpus cavernosum (HCC) obtained by a standardized surgical procedure (14), we have demonstrated that human penile tissue expresses both CBS and CSE, and tissue homogenates efficiently convert L-Cys to H2S. Functional studies, performed in vitro, confirmed that the L-Cys/H2S pathway plays a functional role in human tissue. Indeed, either sodium hydrogen sulfide (NaHS), an exogenous source of H2S, or L-Cys, the substrate for CBS/CSE, relaxed HCC strips in a concentration-related manner. Pharmacological modulation of CBS and CSE by using a CSE inhibitor [propargylglycin (PAG)] and/or a CBS inhibitor [aminooxyacetic acid (AOAA)] confirmed the involvement of the L-Cys/H2S pathway both in vitro and in vivo in rats. The intracavernous administration of either NaHS or L-Cys to rats elicited penile erection, and the response to L-Cys was blocked by PAG. Collectively, these observations indicate that a functional L-Cys/H2S pathway may be involved in mediating penile erection in humans and other mammals.
KCl-induced precontraction (80 mM, Fig. 3). In the presence of stable tone elicited by phenylephrine (PE; 3 μM), the H₂S donor, NaHS (1 μM–10 mM), caused a concentration-dependent relaxation in an endothelium-independent manner (Fig. 3A). To assess further the potential involvement of endogenous NO in the NaHS effect, the strips were incubated with 100 μM L-NAME. L-NAME slightly reduced NaHS-induced relaxation but only at the highest NaHS concentration (Fig. 3A). Then, to investigate further the underlying mechanism involved in the relaxation caused by NaHS, we precontracted HCC with U46619 (10 nM) or h-endothelin-1 (h-ET1, 30 nM), 2 known modulators of the Rho kinase pathway. In this setting, the NaHS-induced relaxation was robustly enhanced in HCC precontracted with U46619 compared with the results obtained with HCC precontracted with PE (Fig. 3B). Similarly, in the presence of h-ET1, we observed a significant increase in NaHS-induced relaxation (Fig. 4B). In contrast, the relaxant effect mediated by NaHS was inhibited by KCl-induced precontraction (80 mM, Fig. 3B). Additionally, glibenclamide (GLB, 150 μM), a Kₐᵥ channel inhibitor, significantly attenuated the relaxation caused by NaHS in HCC strips precontracted with 3 μM PE (Fig. 3C). Fig. 3F reveals that Ach relaxed HCC strips only in the presence of endothelium.

**Effect of L-Cys in HCC Strips.** To verify the involvement of the L-Cys/H₂S pathway in HCC, we challenged the strips with L-Cys. L-Cys relaxed HCC strips in an endothelium-independent manner (Fig. 3D). AOAA (1 mM) incubation significantly inhibited L-Cys-induced relaxation (Fig. 3E).

**HCC Strips and Electric Field Stimulation (EFS).** EFS elicited contraction of HCC strips under resting conditions. Addition of the vehicle did not modify the contraction at 30 or 60 min. Incubation with 10 mM PAG (Fig. 4A) or 1 mM AOAA (Fig. 4B) at
endothelium. (d’Emmanuele di Villa Bianca et al. PNAS)

NaHS Increases Intracavernosal Pressure in Anesthetized Rats. To evaluate whether NaHS causes penile erection in vivo, we monitored the intracavernous pressure after NaHS administration into the rat corpus cavernosum. To validate the experimental procedure, we used acetylcholine (Ach, 50, 100 or 500 µg per rat), which caused a dose-dependent increase in intracavernous pressure (Fig. 5A). NaHS at doses of 10, 30, or 100 µg per rat induced a significant increase in intracavernous pressure as shown in Fig. 5A. The NaHS administration at a lower dose (10 µg per rat) failed to modify the mean arterial blood pressure, whereas the higher doses tested (30 and 100 µg per rat) caused a small dose-unrelated but significant increase in mean arterial blood pressure (Fig. 5C). Ach caused a dose-dependent decrease in mean arterial blood pressure (Fig. 5C). Injection of 50 µL of saline (vehicle) caused no appreciable effect (Fig. 5).

Intracavernous administration of L-Cys (30 µg per rat) caused a significant increase in intracavernous pressure (Fig. 5B). Intravenous administration of PAG (50 mg/kg) at 30 and 60 min significantly reduced L-Cys elicited rat penile erection (Fig. 5B). These data support the possibility that H$_2$S is a natural mediator of penile erection in the rat.

Discussion

H$_2$S is a new emerging gaseous signaling molecule (15–17). H$_2$S is normally present in humans and can be generated endogenously from L-Cys in a reaction catalyzed by either CBS or CSE. Historically, H$_2$S was known as a toxic gas, and mammalians have evolved a fine-tuned regulatory system to control H$_2$S generation at the cellular level by catabolism of the gas through oxidation in mitochondria or by methylation followed by scavenging by metalloproteins and heme-containing compounds (18). The role of H$_2$S in vascular homeostasis is a new concept, and in recent years data have accumulated suggesting a vasorelaxant role for H$_2$S (15–17, 19), and a possible cross-talk with the NO pathway has been proposed (20). H$_2$S was recently shown to be a physiologic vasorelaxant in mice with deletion of CSE (19).

In penile erection there is a strong involvement of the vascular system, and the L-Arg/NO pathway plays a major role (21–24). Following the parallelism between NO and H$_2$S recently established in other cardiovascular areas, we addressed the question of whether H$_2$S could act as a mediator in HCC and thus be involved in human erectile function. Previous studies have indicated the possible involvement of this gaseous mediator in rodents and monkeys (12, 13). However, there are no published studies using human tissue. To address this issue, we used HCC obtained from patients undergoing sex change, which we have already shown to be a reliable tissue source to study receptors and mediators involved in human physiology (14, 25–27). Human tissue expressed CBS and CSE both as protein and mRNA. The quantitative (q) RT-PCR studies clearly showed that mRNA levels of CBS and CSE are similar in HCC. Next, we addressed the question of the tissue localization of both enzymes. HCC tissue stained strongly for CSE and to a lesser extent for CBS. Interestingly, a clear immunoreactivity for CSE and CBS was detected in bundles of muscular tissue and in trabeculae. Immunoreactivity for CSE was also observed in the vascular smooth-muscle cells of penile vessels and in the penile artery. These data are in line with the current belief that CSE rather than CBS is more important in H$_2$S production at the vascular level (28). In addition, peripheral nerves showed a positive stain...
to CSE, in contrast to CBS, suggesting that CSE may modulate the L-Cys/H$_2$S pathway in peripheral cavernous nerves of man. Next, we demonstrated that HCC tissue homogenates under basal conditions produces ≈0.3 nmol of H$_2$S per mg of protein and that addition of exogenous L-Cys boosts the production of H$_2$S by 3-fold. The specificity of the assay and the involvement of both CBS and CSE were confirmed by the finding that H$_2$S production was prevented by either PAG, an inhibitor of CSE, or AOAA, an inhibitor of CBS. These data indicate that HCC can synthesize H$_2$S from L-Cys via the catalytic actions of CBS and CSE.

Having determined that (i) HCC possesses both CBS and CSE protein and mRNA, (ii) homogenates of HCC convert L-Cys (the substrate) to H$_2$S, (iii) PAG and AOAA inhibit L-Cys conversion, and (iv) both enzymes are localized in the smooth-muscle and vascular component of the corpus cavernosum, we performed a functional study by using isolated HCC strips. EFS of HCC strips, under resting conditions, caused a contraction that increased in a frequency-dependent manner. Subtraction of the H$_2$S component, by incubation of HCC strips with PAG or AOAA, produced a significant increase in EFS-induced contraction, confirming the involvement of the L-Cys/H$_2$S pathway in maintaining basal tone. In line with this finding, HCC strips precontracted with PE relaxed to L-Cys in an endothelium-independent and concentration-dependent manner, and this effect was significantly inhibited by AOAA. The capacity of HCC strips to respond to H$_2$S was also confirmed by the fact that HCC strips relaxed in a concentration-dependent manner to NaHS, an H$_2$S donor. This effect was endothelium-independent, but at the higher concentration of NaHS tested, there was a significant inhibition of HCC relaxation by L-NAME. This effect may or may not be important. Because it has been suggested that there is a possible cross-talk between NO and H$_2$S (29), all of the other experiments in vitro, using NaHS, were performed by using HCC strips without endothelium present.

To gain further insight into the possible mechanism(s) underlying the H$_2$S effect, the HCC strips were precontracted with h-ET1 or U46619, a stable analog of thromboxane. These 2 stimuli were selected because the contractile mechanisms they trigger strongly rely on activation of the Rho kinase pathway (30, 31). RhoA is a monomeric GTPase that is inactive when GDP is bound but becomes active after binding to GTP. Activated RhoA stimulates Rho kinase, a serine/threonine kinase. Rho kinase phosphorylates the myosin light chain (MLC) phosphatase at the myosin-binding subunit, thereby causing its inactivation. This results in an increased expression of phosphorylated MLC leading to myosin binding to $\alpha$-actin and smooth-muscle contraction. This pathway is particularly relevant in the erectile mechanism(s) because selective inhibition of the RhoA/Rho kinase pathway has been shown to promote erectile responses in the rat (32). When NaHS was used to relax HCC strips contracted with either U46619 or h-ET1, there was a marked increase in the H$_2$S vasorelaxant effect, suggesting that H$_2$S may interfere with this contractile mechanism. Interestingly, when HCC strips were precontracted with KCl there was a marked inhibition of the NaHS vasorelaxant effect, suggesting that part of the action could be mediated by potassium conductance channels also suggested by the effect glibenclamide, an inhibitor of K$_{ATP}$ channels (1). Experiments using CBS knockout mice were not considered because they have serious hyperhomocysteinemia (40- to 50-fold elevated plasma homocysteine) and need to be treated with homocysteine-lowering drugs to survive a reduced life span (33–36). These mice have growth retardation, reduced survival and altered vascular responsiveness to cholinergic and bradykinin stimulation, making their use of questionable value in the present experiments. During revision of this manuscript, a study in which CSE knockout mice were developed revealed that such mice had much reduced serum H$_2$S levels, developed age-related hypertension, and showed vasorelaxant effects to administered H$_2$S (19). Such mice were fertile, which may be attributed to a more dominant NO-mediated erectile system or development of alternate pathways for erectile function.

The present in vitro data strongly support the case for a role of H$_2$S as a natural signaling molecule in HCC. However, the question of whether the L-Cys/H$_2$S pathway is involved in penile erection in vivo needed to be addressed. In vivo animal models of measuring the cavernous pressure in the rat or mouse have been widely used to study the effects of phosphodiesterase 5 inhibitors and to define the relevance of the other pathways in penile erection (32, 37). When we challenged anesthetized rats with NaHS, there was a dose-dependent increase in intracavernous pressure, implying that exogenous H$_2$S can cause penile erection. In parallel, we performed experiments with acetylcholine to be certain that the experimental model used does respond to a well-established physiological exogenous stimulus. Interestingly, whereas administration of acetylcholine caused, as ex-
pected, a systemic hypotension, NaHS caused a small but not dose-dependent hypertension. This slight hypertension is diffi-
cult to explain because i.v. or intraarterial administration of H2S
should cause hypotension (4). A possible explanation could be
related to the observation that low concentrations of NaHS
tested in vivo cause an increase in vascular smooth muscle contraction
followed by relaxation at higher concentrations (29). To address
further the role of this pathway in penile erection, we sought to
determine whether the intracavernous injection of L-Cys could
cause a change in intracavernous pressure. L-Cys increased the
intracavernous pressure, and this effect was prevented by in vivo
administration of PAG. These data indicate that L-Cys elicits an
erectile response in rats that is blocked by an inhibitor of H2S
formation from L-Cys.

In conclusion, the data presented in this study demonstrate
collectively that the L-Cys/H2S signaling pathway is involved in
mediating HCC smooth-muscle relaxation. Therefore, it is pos-
ted that H2S may function to mediate penile erection in
humans, as it appears to do in rats. To what extent this pathway
complements the L-Arg/NO signaling pathway in promoting
erectile function is presently unknown. These observations may
help to unravel the complex mechanisms underlying the patho-
physiology of human penile erection and may lead to the
development of therapeutic approaches in the treatment of ED
and sexual arousal disorders.

Materials and Methods

Human Tissue. In male-to-female transsexual surgical procedures, the penis
and testicles are amputated, and a neovagina is created to simulate female
external genitalia. Patients undergo appropriate hormonal pretreatment
with antiandrogens and estrogens to adapt to female appearance, and the
therapy is discontinued 2 months before surgery. The corpora cavernosa
were carefully excised from the penis immediately after amputation and placed in
ice-cold oxygenated Krebs solution and washed extensively with heparinized
Kreb's solution. After the laveage, the corpora cavernosa were placed in ice-cold
Kreb's solution and kept on ice until the experiments were conducted (14).
All patients were informed of all procedures and gave their written consent.
The protocol was approved by the Ethics Committee of the Medical School of the
University of Naples Federico II. Corpus cavernosum specimens were obtained from
6 different individuals.

Real-Time Quantitative RT-PCR. The presence of CBS and CSE was determined
by PCR. Total mRNA from HCC was extracted by using TRIzol reagent (Invitro-
gen, according to the manufacturer’s recommendations). Reverse transcription
was performed, and 100 ng of the RNA samples described above was used for
qPCR. Samples were run in triplicate in 50-μL reactions using an ABI PRISM 5700 sequence detector system (Applied Biosystems). Samples were incubated at 42 °C for 5 min and 95 °C for 10 min, followed by 40 cycles at 95 °C
for 15 s and 60 °C for 1 min. SYBR Green oligonucleotides to detect human CBS
and CSE were specifically designed by using primer express software (Applied
Biosystems) and validated for their specificity. Relative quantification of tar-
get cDNA was performed by arbitrarily setting the control value at 100, and
changes in cDNA content of a sample were expressed as a multiple thereof.
Differences in cDNA input were corrected by normalizing signals obtained
for qPCR. Samples were run in triplicate in 50-
μL reaction mixture (total volume 500
μL) containing 20 μL of 2× SYBR Green master mix and 0.3 μM primers specific for GAPDH. mRNA copy differences were corrected by
differences in cDNA content of a sample were expressed as a multiple thereof.

Western Blotting. Western blotting was performed in modified RIPA buffer (50 mM
Tris·HCl (pH 7.4), 1% Triton X-100, 0.25% sodium deoxycholate, 150 mM NaCl,
1 mM EDTA, 1 mM PMSF, 10 μg/mL aprotinin, 20 μg/mL leupeptin, 50
mMol/L NaF) by using a polymeron homogenizer (2 cycles of 10 s at maximum
speed). After centrifugation of homogenates at 10,000 rpm for 10 min, 10–30 μg
of the denatured proteins were separated on 10% SDS/polyacrylamide gels and
deposited to a PVDF membrane. Membranes were blocked by incuba-
tion in PBS containing 0.1% vol/vol Tween 20 and 5% nonfat dry milk for 2 h,
followed by overnight incubation at 4 °C with mouse polyclonal CBS (Abnova
Novus Biologicals) antibody (1:500) or mouse monoclonal CSE (Abnova
Novus Biologicals) antibody (1:500). The filters were washed extensively in PBS
containing 0.1% vol/vol Tween 20 before incubation for 2 h with anti-
primers for CBS and CSE. Anti-CBS and -CSE were specifically designed by using primer express software (Applied
Biosystems) and validated for their specificity. Relative quantification of tar-
get cDNA was performed by arbitrarily setting the control value at 100, and
changes in cDNA content of a sample were expressed as a multiple thereof.
Differences in cDNA input were corrected by normalizing signals obtained
for qPCR. Samples were run in triplicate in 50-
μL reaction mixture (total volume 500
μL) containing 20 μL of 2× SYBR Green master mix and 0.3 μM primers specific for GAPDH. mRNA copy differences were corrected by
differences in cDNA content of a sample were expressed as a multiple thereof.

Immunohistochemistry. HCC samples were snap frozen in liquid nitrogen
in OCT embedding medium (Tissue Tek) and stored at −80 °C or fixed overnight
in 4% buffered formalin and paraffin-embedded. Cross-sections were cut (6
μm) and used for CSE and CBS detection by immunohistochemistry. Paraffin
sections, after being dewaxed and rehydrated, were boiled for 30 min in
citrate buffer for antigen retrieval. Cryostat sections were incubated in ace-
tone for 10 min, air dried, and rehydrated with PBS. For staining, sections
were incubated with 3% H2O2 in methanol for 10 min, and protein block serum-free
(blocking solution) was added for 15 min. Sections were washed with PBS
and transferred to a PVDF membrane. Membranes were blocked by incuba-
tion in PBS containing 0.1% vol/vol Tween 20 before incubation for 2 h with anti-
primers for CBS and CSE. Anti-CBS and -CSE were specifically designed by using primer express software (Applied
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Differences in cDNA input were corrected by normalizing signals obtained
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μL reaction mixture (total volume 500
μL) containing 20 μL of 2× SYBR Green master mix and 0.3 μM primers specific for GAPDH. mRNA copy differences were corrected by
differences in cDNA content of a sample were expressed as a multiple thereof.

Measurement of H2S in HCC. H2S determination was performed according to
Stipanuck and Beck (37) with modifications. The tissue was homogenized in a
lysis buffer [100 mM potassium phosphate buffer (pH 7.4), 10 mM sodium
orthovanadate, and protease inhibitors]. Protein concentration was deter-
mained by using the Bradford assay (Bio-Rad). Homogenates were added in a
reaction mixture (total volume 930 μL). The reaction was started with piridoxal
5'-phosphate, 20 μL of 10 mM L-Cys, and 30 μL of saline. The reaction was performed in parafilm Eppendorf tubes and initiated by transferring tubes
from ice to a water bath at 37 °C. After incubation of 30 min, 250 μL of 1%,
Zn(Ac)2, was added followed by 250 μL of 10% trichloroacetic acid. Subse-
quently, 133 μL of 20 mM N,N-dimethyl-L-phenylisodiamine-sulphate (DPD)
in 7.2 M HCl and 133 μL of 30 mM FeCl3 in 1.2 M HCl were added, and the
absorbance of the solution was measured after 20 min at a wavelength of
650 nm. The H2S synthesis inhibitors PAG (10 mM), AOA (1 mM), or a combina-
tion of both were added 5 min before addition of L-Cys. All samples were assayed
duplicate, and H2S concentrations were calculated according to a calibration
curve of NaHS (3.12–250 μM). Results were expressed as nmoles per milligram
of protein·min−1 and calculated as mean ± SEM from 4 specimens. Data were
analyzed using Student's t test.

HCC Strips. Longitudinal strips (2 cm) of HCC were dissected from the trabecu-
lar structure of the penis and isolated (14). Krebs solution had the following
composition: 115.3 mM NaCl, 4.9 mM KCl, 1.46 mM CaCl2, 1.2 mM MgSO4, 1.2
mM KH2PO4, 25.0 mM NaHCO3, 11.1 mM glucose (Carlo Erba). HCC strips were
incubated in a 2 mL organ bath containing oxygenated (95% O2 and 5% CO2)
Krebs solution at 37 °C. HCC strips were connected to isometric force-
displacement transducers (Biology, Ugo Basile) and were immersed in Krebs
solution, recorded continuously by using a polygraph linearecorder (WR3310;
Graphite). Tissues were preloaded with 2 g of tension and allowed to equi-
librate for 90 min in Krebs solution that was changed at 15-min intervals. After
equilibration, tissues were standardized by performing repeated 3 μM PE
(Sigma) contractions until 3 equal responses were obtained. After standard-
ization, endothelial integrity was assessed by using 0.10–10 μM Ach (Sigma)
(see Fig. 3F). Strips without a functional (Ach-responsive) endothelium were
obtained by incubating in distilled water for 15 s. A concentration–response
curve to 1 μM–10 mM NaHS (Sigma) was obtained in the presence or absence
of endothelium, by using HCC strips precontracted with 3 μM PE. To assess the
involvement of NO, we incubated the strips for 20 min with 100 μM l-NAME
(Sigma) before NaHS challenge. KATP Channel involvement (4) in NaHS-induced
relaxation was assessed by incubating HCC-denuded strips with 150 μM
glibenclamide (Sigma) for 20 min. In another set of experiments, HCC strips
without endothelium were precontracted with 10 mM U46619 (Alexis), 30 mM
L-ET1 (Tocris) or 80 mM potassium chloride (Carlo Erba). In another set of

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and challenged with 1 using HCC strips with or without endothelium. In another set of experiments, a concentration–response curve to 1 responses to EFS were expressed as force in dynes per milligram of tissue. This contractile response was repeated after 30 and 60 min of 

parameters of 5, 10, 20, and 40 Hz (frequency), 1 ms (pulse width), and 90 V (amplitude). This contractile response was repeated after 30 and 60 min of incubation with 10 mM PAG or 1 mM AOAA or vehicle (saline). The tissue responses to EFS were expressed as force in dynes per milligram of tissue. Experiments were performed on 3 different specimens. Finally, a concentration–response curve to 1 was obtained by using HCC strips with or without endothelium. In another set of experiments, in the absence of endothelium, the strips were incubated with 1 mM AOAA and challenged with a 1 μM–10 mM L-Cys. Data were analyzed by using ANOVA followed by Bonferroni’s post hoc test. The data present the mean ± SEM from 8 separate specimens for NaHS or 5 for L-Cys.

### Monitoring Intracavernous Pressure in Anesthetized Rats

The present work was performed in accordance with the guidelines of Italian law (No. 116/1992) and European Council law (No. 86/609/CEE) for animal care. Male Wistar rats weighing 200–250 g were used (Charles River). Animals were kept under laboratory conditions (temperature 23 ± 2 °C, humidity range 40–70%, 12-h light/dark cycle). Food and water were fed ad libitum. Rats were anesthetized with an i.p. injection of urethane (1 g/kg), so that the rats breathed spontaneously during the experiment. For continuous systemic blood pressure measurements, a heparinized (5 units/mL) polyethylene catheter was inserted into the carotid artery connected to a pressure transducer (BLPR-2; 2Biological Instruments). With a midline perineal incision, followed by blunt dissection of the overlying straited muscles, entrance to the tunica albuginea of the crus corpus cavernosum was achieved. A 26-gauge needle attached to a heparinized (50 units/mL) polyethylene catheter was inserted into the crus corpus cavernosum, and the intracavernous pressure was monitored with a pressure transducer (BLPR-2). These parameters were recorded, and data acquisition and calculations were performed by using a computer system (Biopac; 2Biological Instruments). For pharmacological evaluation via the intracavernous route, a 26-gauge needle was placed at the other crus for drug injection. NaHS dissolved in 50 μL of saline was given at doses of 10, 30, and 100 μg per rat. To validate the experimental model we used Ach in 50 μL of saline at doses of 50, 100, and 500 μg per rat. In another set of experiments we assessed the involvement of H2S in penile erection by using L-Cys in 50 μL of saline at a dose of 30 μg per rat. Saline (50 μL) served as the control vehicle. L-Cys was injected intracavernously, and PAG (CSE inhibitor) was administered at a dose of 50 mg/kg i.v. (30- and 60-min pretreatment). Data were calculated as area under the curve (mmHg × min) and expressed as mean ± SEM from 7 separate experiments for NaHS and 4 for L-Cys. The changes in systemic blood pressure were calculated as differences from basal values after intracavernous drug injection (mmHg) and expressed as mean ± SEM. Data were analyzed by using Student’s t-test.

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