Decreased Endogenous Hydrogen Sulfide Generation in Penile Tissues of Diabetic Rats With Erectile Dysfunction

Yan Zhang, MS,1,2 Jun Yang, MD, PhD,1,2 Tao Wang, MD, PhD,1,2 Shao-Gang Wang, MD, PhD,1,2 Ji-Hong Liu, MD, PhD,1,2 Chun-Ping Yin, MS,3 and Zhang-Qun Ye, MD, PhD1,2

ABSTRACT

Introduction: Hydrogen sulfide (H2S) is an endogenous gasotransmitter. The levels of H2S-generating enzyme expression and endogenous H2S production in diabetic rats with erectile dysfunction (ED) remain unknown. The aim of this study was to investigate the expression of the H2S-generating enzymes and endogenous production of H2S in penile tissues of diabetic ED rats.

Methods: Experimental rats were randomly divided into normal control group, apomorphine (APO)-positive group and APO-negative group. Primary rat corpus cavernosum smooth muscle cells (CCSMCs) and aortic endothelial cells (AECs) were isolated and cultured in vitro under 3 different conditions: normal glucose (NG) condition, high glucose (HG) condition, and osmotic control (OC) condition.

Main Outcome Measures: Erectile function; H2S concentrations in plasma or penile tissues; expression of H2S-generating enzymes and endogenous H2S production in penile tissues, CCSMCs, and AECs.

Results: Erectile function was significantly decreased in the APO-negative group. In addition to significantly decreased expression of cysteine aminotransferase (CAT), D-amino acid oxidase (DAO), and 3-mercaptopyruvate sulfurtransferase (3-MST), the H2S concentrations in plasma and penile tissues and endogenous H2S production were significantly decreased in the APO-negative group. Endogenous H2S production by cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) decreased to the same levels in the APO-negative and APO-positive groups as that in the normal control group. However, CBS and CSE expression remained unchanged in the 3 groups. Under HG conditions, H2S-generating enzyme expression in AECs did not change, while CAT, DAO, and 3-MST expression in CCSMCs was significantly decreased. In both cell types, H2S production by these enzymes was decreased in the HG group.

Conclusion: Endogenous H2S production was significantly decreased in the diabetic ED rats’ penile tissues due to downregulated expression of the CAT/3-MST and DAO/3-MST pathways and low activities of CBS and CSE.


Key Words: Hydrogen Sulfide; Erectile Dysfunction; Penile Tissues; Corpus Cavernosum Smooth Muscle Cells; Aortic Endothelial Cells

INTRODUCTION

Erectile dysfunction (ED) is one of the most common complications of diabetes mellitus (DM). Epidemiological studies have reported that more than 50% of men with DM suffer from ED.1 Additionally, ED in men with DM is more severe than that in the general population, leading to a worse quality of life.2 Currently, phosphodiesterase 5 (PDE5) inhibitors are used as the first-line drug treatment for ED. However, this treatment is less effective in diabetic men with ED than in men with other types of ED.3 Thus, it is necessary to explore new therapeutic targets for diabetic ED.

Hydrogen sulfide (H2S), a well-known toxic gas, has emerged as the most important regulator of physiological and pathophysiological conditions in vivo, along with nitric oxide (NO) and carbon monoxide (CO). H2S is mainly synthesized in mammalian cells by 3 enzymes—cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST)—all of which are expressed in many
cells of the mammalian body. Both CBS and CSE use L-cysteine as a substrate to form H₂S in the presence of pyridoxal-5-phosphate (PLP). Recently, another pathway that is not PLP dependent has been discovered. In this pathway, 3-MST produces H₂S from 3-mercaptopyruvate (3-MP), which in turn is produced by cysteine aminotransferase (CAT) and from D-cysteine by D-amino acid oxidase (DAO). Endogenous and exogenous H₂S exhibit protective effects by neutralizing reactive oxygen species (ROS), promoting vascular smooth muscle relaxation, reducing apoptotic signaling, and reversibly modulating mitochondrial respiration, which is mediated by different molecular targets, including various ion channels and signaling proteins, indirectly suggesting the probable role of H₂S in penile erectile function.

Until recently there has been little evidence suggesting that H₂S exerts important biophysiological effects on erectile function. Both CSE and CBS have been found in the smooth muscle components of the human corpus cavernosum, whereas only CSE is expressed in the dorsal nerve. Administration of exogenous sodium hydrogen sulfide significantly increases penile length and cavernous pressure, whereas inhibition of CSE and CBS result in contraction of the corpus cavernosum and a decrease in the normal intracavernous pressure (ICP) response to electrostimulation, suggesting a role of H₂S in smooth muscle relaxation in penile tissues. In addition, exogenous H₂S may exert its proerectile effects by augmenting the NO pathway. However, the abovementioned studies all have examined the effects of exogenous or endogenous hydrogen sulfide on normal penile tissues, and limited information has been reported on the endogenous H₂S system in diabetic ED. The aim of this study was to evaluate the possible changes in the endogenous H₂S system in penile tissues of diabetic ED rats through in vivo and in vitro experiments.

MATERIALS AND METHODS

Experimental Animals

All procedures involving animals were conducted in accordance with the guidelines of the Chinese Council on Animal Care and with approval from the Committees on Animal Experiments at Tongji Hospital (Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China). Fifty-nine 45-day-old Sprague-Dawley rats were included in this study. Nine were used for the primary cell culture, while 7 were randomly selected as the normal control group. The other animals were used to induce type 1 diabetes by intraperitoneal injection of 60 mg/kg streptozotocin (STZ; Sigma-Aldrich, St Louis, MO, USA), as previously reported. At 72 hours after STZ injection, animals were considered diabetic if their blood glucose concentration was higher than 16.7 mmol/L. After 8 weeks, apomorphine (APO; Sigma-Aldrich) was used to identify the diabetic ED rats, as previously reported. Briefly, the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg) followed by cannulation of the left carotid artery with PE-50 tubing (Intramedic, Franklin Lakes, NJ, USA) to facilitate continuous

Rat Corpus Cavernosum Smooth Muscle Cell Isolation and Culture

Rat corpus cavernosum smooth muscle cells (CCSMCs) were isolated and cultured from corpus cavernosum tissues as previously described by our group. Briefly, corpus cavernous tissues were obtained from 8-week-old rats. They were minced into cubes of approximately 1 mm³ in size and placed in an overturned 25 cm² culture flask, followed by addition of a small amount of low-glucose Dulbecco’s modified Eagle’s medium (DMEM) (Thermo Fisher, Waltham, MA, USA). The overturned flask was incubated in a humidified 5% CO₂–95% air incubator at 37°C for half an hour. Then the flask was turned upright and the tissues were submerged in culture medium. CCSMCs migrated from the explants and were purified by differential adherence velocity. Immunostaining with an anti—α-smooth muscle actin (α-SMA) antibody (1:200, Boster, Wuhan, China) was used to identify CCSMCs.

Rat Aortic Endothelial Cell Isolation and Culture

Rat aortic endothelial cells (AECs) were prepared from the thoracic aortas of 8-week-old male rats using the previously described explant method. Briefly, rat thoracic aortas were cut into approximately 1-mm² sections and placed with the intimal side down into T25 flasks. Endothelial growth medium-2-MV (EGM; Lonza, Walkersville, MD, USA) was gassed with 5% CO₂ to maintain the same osmotic pressure as that of the HG condition. Each culture medium was changed every 12 hours to maintain the glucose concentration and osmotic pressure.

In Vivo Evaluation of Erectile Function

The erectile function of each rat in response to electrical stimulation was assessed by our team members as previously described. Briefly, the rats were anesthetized by intraperitoneal injection of pentobarbital sodium (40 mg/kg) followed by cannulation of the left carotid artery with PE-50 tubing (Intramedic, Franklin Lakes, NJ, USA) to facilitate continuous
measurement of the mean systemic artery pressure (MAP). A 25-gauge needle was inserted into the right crura of the penis to continuously monitor the ICP. The pressure data were recorded continuously with a data acquisition system (AD Instruments Powerlab/4SP, Bella Vista, NSW, Australia). Stimulation of the cavernous nerve was performed at a 15-Hz frequency for 1 minute, and the erectile response was measured at 5.0 volts, with 3-minute intervals between stimulations. The ratio of the maximal ICP to the MAP was calculated to evaluate erectile function.

Real-time Quantitative PCR Analysis

Total RNA was extracted from penile tissues or cultured cells using a Multisource RNA Miniprep Kit (Corning Inc, Corning, NY, USA). cDNA was synthesized from 500 ng RNA using a PrimeScript RT Reagent Kit (TaKaRa, Dalian, CA, USA) and SYBR Premix Ex Taq (TaKaRa). The sequences of the primers used in real-time quantitative PCR are listed in Table 1. Amplification specificity was evaluated using a melting curve. The β-actin mRNA expression level was used as an endogenous reference and for normalization purposes. Relative quantification of the expression level of each transcript for each group was calculated using the $2^{-\Delta\Delta CT}$ method.

Western Blotting Analysis

Penile tissues or cultured cells were homogenized, and total, cytoplasmic, and nuclear proteins were isolated using NP40 or a nuclear and cytoplasmic protein extraction kit (Beyotime, Nan- tong, China). Equal amounts of protein (50 µg/lane) were electrophoresed on 12% sodium dodecyl sulfate—polyacrylamide gels and transferred to polyvinylidene difluoride membranes. The membranes were blocked and then incubated with the following primary antibodies: rabbit anti-CBS (1:1000, Proteintech), rabbit anti-CSE (1:1000, Proteintech), rabbit anti-CAT (1:1000, Proteintech), rabbit anti-DAO (1:1000, Affinity, Zhenjiang, China), and mouse anti−3-MST (1:500, Santa Cruz, CA, USA). Protein bands were detected by horseradish peroxidase conjugated secondary antibodies (1:5000, Proteintech).

### Table 1. List of Primer Sequences Used in Real-Time Quantitative PCR Analysis

<table>
<thead>
<tr>
<th></th>
<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>β-Actin</td>
<td>GGAGATTACTGGCCCTGGCTCCTCA</td>
<td>GACTCATCCTACTCCTGTTGCTG</td>
</tr>
<tr>
<td>CAT</td>
<td>GGACAACGCCAGCTCTCTCA</td>
<td>CATTGTTACATCAGCCCDTAAGAA</td>
</tr>
<tr>
<td>CBS</td>
<td>AAGGCTGCCAGAGCTAAAG</td>
<td>CACATCCATTTGTCACTCAAGAAC</td>
</tr>
<tr>
<td>CSE</td>
<td>CACCCGGAGCCAATGGAG</td>
<td>GAGTTTCAGAGGCGGCTGTA</td>
</tr>
<tr>
<td>DAO</td>
<td>AGCCCCAGATCAGAGGCAAT</td>
<td>ATGACTCCCGCTC4AACATC</td>
</tr>
<tr>
<td>3-MST</td>
<td>TTCATCAAGACGCCAGAGGATA</td>
<td>TTCACTGGAATGGATGTTACTGAG</td>
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Measurement of Plasma and Penile Tissue H$_2$S Concentrations

The H$_2$S concentrations in plasma and penile tissues were measured using the methylene blue method, according to a previous study. Plasma samples (200 µL) or tissue homogenates were transferred to Eppendorf tubes. Two hundred fifty microliters of zinc acetate (1%) was added, followed by injection of 133 µL N,N-dimethylphenylenediamine sulfide (20 mmol/L) in 7.2 mol/L HCl, and 133 µL FeCl$_3$ (30 mmol/L) in 1.2 mol/L HCl. Finally, the absorbance of the supernatant fraction was determined at a wavelength of 670 nm. All of the chemicals for H$_2$S measurement were purchased from Sigma-Aldrich.

Analysis of Endogenous H$_2$S Synthesis

Endogenous production of H$_2$S by penile tissue homogenates or cellular proteins was measured using the methylene blue method with slight modifications. In brief, penile tissues or cells were homogenized in a 10-fold volume of ice-cold 50 mmol/L Tris-HCl buffer. The tissue homogenates or cellular proteins (0.1 mL) were incubated at 37 °C in the presence or absence of reaction buffer. After 90 minutes of incubation, the H$_2$S concentration was determined with the methylene blue method.

Endogenous H$_2$S synthesis was induced using different reaction buffers. Reaction buffer I (to measure H$_2$S produced by CBS and CSE) contained L-cysteine (10 mmol/L), L-homocysteine (10 µmol/L), PLP (2 mmol/L), and L-aspartic acid (20 mmol/L) at pH 7.4 in 50 mmol/L Tris-HCl buffer. Reaction buffer II (to measure H$_2$S produced by CAT/3-MST and DAO/3-MST) contained L-cysteine (10 mmol/L), PLP (2 mmol/L), α-ketoglutarate (2.5 mmol/L), d-cysteine (10 mmol/L), and trifluoroacetic acid (0.7 mmol/L) at pH 8.0 in 50 mmol/L Tris-HCl buffer.

Statistical Analysis

The data are all presented as the mean ± standard deviation (SD). Statistical significance was assessed by Student t test for comparisons of 2 groups or 1-way analysis of variance followed by the post hoc (Tukey) test for comparisons among multiple groups. Intergroup differences were considered statistically significant at a P value of < .05.
Experimental Groups After 8 Weeks

Among the 3 groups. Furthermore, we attempted to determine compared with those in the normal control group (decreased in the APO-negative and APO-positive groups (Figure 1B). Thus, the APO-negative group was composed of diabetic rats with severe ED. When we compared the normal control and APO-negative groups, the latter group showed a remarkable decrease in the ICP/MAP ratio (P < .05) (Figure 1B). Thus, the APO-negative group was composed of diabetic rats with severe ED.

H₂S-Generating Enzyme Expression in Penile Tissues

As shown in Figure 2A, CSE, CBS, CAT, DAO, and 3-MST mRNA all were expressed in the penile tissues. The relative expression levels of CAT, DAO, and 3-MST were significantly decreased in the APO-negative and APO-positive groups compared with those in the normal control group (P < .05), and there were significant differences in these levels between the APO-positive and APO-negative groups (P < .05). However, the mRNA expression of CSE and CBS did not change significantly among the 3 groups. Furthermore, we attempted to determine the mRNA expression of these H₂S-generating enzymes in normal control rats’ penile tissues by normalizing their expression to that of CSE. As shown in Figure 2B, the mRNA expression levels of CBS and DAO was decreased (0.67- and 0.27-fold, respectively; P < .05) compared with that of CSE, whereas the expression levels of CAT and 3-MST were much higher (2,834- and 4,872-fold, respectively; P < .05).

Consistent with the real-time quantitative PCR results, the Western blotting analysis showed that the CAT, DAO, and 3-MST protein levels were significantly decreased in the APO-negative and APO-positive groups compared with the normal control group (P < .05). CSE and CBS protein expression did not significantly differ among the normal control, APO-positive, and APO-negative groups (Figures 2C and 2D).

H₂S Concentrations in Plasma and Penile Tissues and Endogenous H₂S Generation in Penile Tissues

As shown in Figures 3A and 3B, the H₂S concentrations in the plasma and penile tissues were significantly higher in the APO-negative group compared with those in the APO-negative group (P < .05), but lower than those in the normal control group (P < .05).

Table 2. Body Weights, Fasting Blood Glucose, and MAP of the Experimental Groups After 8 Weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Fasting blood glucose (mmol/L)</th>
<th>MAP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>7</td>
<td>582.9 ± 38.7</td>
<td>6.0 ± 0.5</td>
<td>106.7 ± 6.9</td>
</tr>
<tr>
<td>APO positive</td>
<td>7</td>
<td>270.4 ± 36.8*</td>
<td>27.8 ± 2.1†</td>
<td>1071 ± 7.4</td>
</tr>
<tr>
<td>APO negative</td>
<td>7</td>
<td>201.6 ± 31.5*†</td>
<td>28.5 ± 2.3*</td>
<td>105.0 ± 5.1</td>
</tr>
</tbody>
</table>

*P < .05 vs normal control group.
†P < .05 vs APO-positive group.
We next examined the contribution of these H₂S-generating enzymes to H₂S production in rats’ penile tissues. As shown in Figure 3C, the amounts of H₂S generated by CBS and CSE were decreased in the APO-positive and APO-negative groups compared with that in the normal control group (P < .05). H₂S production by the DAO/3-MST and CAT/3-MST pathways was markedly decreased in the APO-negative group compared with that in the normal control and APO-positive groups (P < .05, Figure 3D).

H₂S-Generating Enzyme Expression Under HG Conditions

CCSMCs and AECs were successfully isolated. CCSMCs were spindle shaped and confirmatory immunostaining with an anti-α-SMA antibody revealed that nearly all CCSMCs expressed α-SMA (Figures 4A and 4B). AECs were cobblestone shaped and were positive for CD31 expression (Figures 5A and 5B). Expression of H₂S-generating enzymes was evaluated in CCSMCs and AECs under the NG, OC, and HG conditions. mRNA expression of all of the H₂S-generating enzymes was unchanged in AECs (Figure 4C). As shown in Figure 5C, different glucose concentrations caused concentration-dependent decreases in CAT, DAO, and 3-MST mRNA expression in CCSMCs (P < .05). CSE mRNA expression in CCSMCs was decreased in both the OC and HG groups to the same level (P < .05), indicating that hyperosmolarity, but not high glucose, resulted in these decreases in expression. DAO was not expressed in AECs, whereas CBS was not expressed in CCSMCs. Likewise, Western blotting analysis showed the same pattern of protein expression as that of mRNA expression (Figures 4D and 5D). All of the H₂S-generating enzymes were distributed in the cytoplasm of CCSMCs and AECs.

Then, endogenous H₂S production in AECs and CCSMCs was analyzed by the methylene blue method. As shown in Figures 4E and 4F, H₂S production by CBS and CSE or via the CAT/3-MST pathway in AECs was significantly decreased...
in the HG group compared with that in the NG and OC groups ($P < .05$), indicating that the activities of all of the H$_2$S-generating enzymes in AECs were decreased in the HG group due to hyperglycemia. Similarly, the amount of H$_2$S produced by CSE or via the CAT/3-MST and DAO/3-MST pathways in CCSMCs was smaller in the HG group compared with that in the NG and OC groups ($P < .05$) (Figures 5E and 5F). However, the amount of H$_2$S produced by CSE in CCSMCs was decreased in the OC group compared with that in the NG group ($P < .05$) due to diminished expression of CSE. CSE was expressed at the same level in CCSMCs under the HG and OC conditions, but it produced less H$_2$S under HG condition. Thus, enzymatic activity of CSE was decreased in the presence of hyperglycemia.

**DISCUSSION**

In our study, we first revealed that all of the H$_2$S-generating enzymes, including CBS, CSE, CAT, DAO, and 3-MST were expressed in rat penile tissues. The H$_2$S concentrations in plasma and penile tissues and endogenous H$_2$S generation were significantly decreased in the APO-negative group. This study demonstrated the expression of CBS and CSE in rat penile tissues using real-time quantitative PCR and Western blotting, consistent with previous studies. CAT/3-MST and DAO/3-MST pathways have recently been receiving increasing attention as parts of a novel H$_2$S-generating system involved in many physiological functions. Nevertheless, to our knowledge, data on CAT/3-MST and DAO/3-MST pathway in penile tissues have not been reported before.
tissues have not been reported to date. In our study, we found that both of these pathways were localized to rat penile tissues, indicating that they may also play important roles in H2S production.

Data on the effect of DM on H2S-generating enzyme expression in penile tissues is rare. In the present study, we revealed that CBS and CSE expression remained unchanged in AECs under the HG condition in vitro and in penile tissues of Figure 4.

Figure 4. Relative mRNA and protein expression of H2S-generating enzymes and H2S production in AECs under different culture conditions. (A) Rat AECs were successfully isolated and cultured (100×). (B) Immunostaining of AECs with an anti-CD31 antibody (100×). (C) H2S-generating enzyme expression was assessed by real-time quantitative PCR, and the results were normalized to β-actin expression using the comparative Ct method. (D) H2S-generating enzyme protein levels in AECs were detected by western blotting analysis, and the graphs show the relative protein levels of the H2S-generating enzymes, which were normalized to the β-actin level. (E) H2S production by CBS and CSE in AECs. Cell homogenates were incubated in reaction buffer I, containing L-cysteine (10 mmol/L), L-homocysteine (10 μmol/L), and L-aspartic acid (20 mmol/L), at 37 °C for 90 min. (F) H2S production via the CAT/3-MST pathways in AECs. Cell homogenates were incubated in reaction buffer II, containing L-cysteine (10 mmol/L), PLP (2 mmol/L), α-ketoglutarate (2.5 mmol/L), D-cysteine (10 mmol/L), and trifluoroalanine (0.7 mmol/L), at 37 °C for 90 min. The data are expressed as the mean ± SD. *P < .05 vs NG group. Figure 4 is available in color online at www.jsm.jsexmed.org.
diabetic ED rats in vivo, consistent with previous studies.\textsuperscript{26,27} However, in vitro CSE expression in CCSMCs under the HG condition was decreased to the same extent as that under the OC condition compared with that under the NG condition, indicating that CSE expression may be regulated by differences in osmotic pressure. Considering that osmotic pressure is almost always constant in the body, a change in CSE expression could not occur. However, several studies have reported different

Figure 5. Relative expression of H\textsubscript{2}S-generating enzymes and H\textsubscript{2}S production in CCSMCs under different culture conditions. (A) Rat CCSMCs were successfully isolated and cultured (100\times). (B) Immunostaining of CCSMCs with an anti-\textalpha-SMA antibody (100\times). (C) H\textsubscript{2}S-generating enzyme expression was assessed by real-time quantitative PCR, and the results were normalized to \textbeta-actin expression using the comparative Ct method. (D) H\textsubscript{2}S-generating enzyme protein levels in CCSMCs were detected by western blotting analysis, and the graphs show the relative protein densities of the H\textsubscript{2}S-generating enzymes, which were normalized to the \textbeta-actin level. (E) H\textsubscript{2}S production by CSE in CCSMCs. Cell homogenates were incubated in reaction buffer I, containing L-cysteine (10 mmol/L), L-homocysteine (10 \textmu mol/L), PLP (2 mmol/L), and L-aspartic acid (20 mmol/L), at 37 °C for 90 min. (F) H\textsubscript{2}S production via the DAO/3-MST and CAT/3-MST pathways in penile CCSMCs. Cell homogenates were incubated in reaction buffer II, containing L-cysteine (10 mmol/L), PLP (2 mmol/L), \textalpha-keto-glutarate (2.5 mmol/L), D-cysteine (10 mmol/L), and trifluoroalanine (0.7 mmol/L), at 37 °C for 90 min. The data are expressed as the mean ± SD. *P < .05 vs NG group; #P < .05 vs OC group. Figure 5 is available in color online at www.jsm.jsexmed.org.
expression levels of CBS and CSE in various tissues from diabetic rats. Suzuki et al. 32 and Denizalti et al. 34 reported no significant changes in the protein expression of CBS and CSE in the artery, heart, lung, kidney, liver, or brain from diabetic animals at 4, 6, or 12 weeks after STZ injection. Yusuf et al. 28 and Nieman et al. 29 found increased mRNA expression of CSE and CBS in livers and of CBS mRNA in pancreases in diabetic rats at 5 days after the induction of diabetes. Wang et al. 30 reported that CBS and CSE protein expression was markedly enhanced in rat aortic tissues at 8 weeks after STZ injection. On the other hand, Yamamoto et al. 31 and Yuan et al. 32 reported decreased CSE expression in kidney tissues from diabetic animals at 8 or 3 weeks after the induction of diabetes, whereas CBS expression was unaffected. Manna et al. 33 showed lower CSE protein expression in liver tissues from diabetic rats at 14 weeks after STZ injection and in peripheral blood mononuclear cells from diabetic patients. Huang et al. 34 found a decrease in CBS and CSE protein expression in penile tissues from diabetic rats at 4 and 6 weeks after STZ injection. Thus, the results of studies regarding CBS and CSE expression are conflicting and additional studies are needed to confirm these results.

CAT/3-MST and DAO/3-MST pathways’ expression was markedly decreased in diabetic ED rats’ penile tissues in vivo. Nevertheless, in vitro expression of all H2S-generating enzymes in CCSMCs, but not in AECs, was altered in accordance with that in penile tissues, indicating that downregulation of the CAT/3-MST and DAO/3-MST pathways in CCSMCs may have contributed to the changes in penile tissues from the diabetic ED rats. Similarly, Coletta et al. 35 reported that 3-MST protein expression in microvascular endothelial cells did not change after 14 days of hyperglycemia. However, the precise mechanism of the decreased expression of these pathways requires further elucidation.

In addition to decreased expression, reduced activities of the H2S-generating enzymes also may play an important role in decreased H2S production. In our study, decreased H2S generation by CBS and CSE in the penile tissues of the APO-negative and APO-positive groups was accompanied by similar expression of both of these enzymes, indicating that their activities could have been affected by hyperglycemia in the diabetic rats. 27,33 The activities of CBS and CSE could be influenced by several endogenous and exogenous factors. CBS-mediated H2S generation in the brain could be enhanced by factors that positively regulate the intracellular Ca2+ and calcium-modulated protein (calmodulin) concentrations, whereas in the cardiovascular system, CSE activity is enhanced by NO donors via a cGMP-dependent mechanism. 36 Studies have demonstrated that both CBS and CSE activity is increased in liver tissues from STZ-induced diabetic rats and in hepatocytes cultured under HG condition. 37,38 However, these studies were conducted in early-stage diabetic rats or cells exposed to HG condition for a short time. CBS is known to be able to irreversibly generate cysteine from homocysteine. The plasma homocysteine level in diabetic patients is reduced in the absence of vascular complications but is increased in the presence of atherosclerosis or reduced renal function, 39 indicating that CBS activity is decreased in late-stage diabetes after long-term exposure to hyperglycemia and oxidative stress.

Our study also has some limitations that merit discussion. We used AECs instead of corpus cavernosal endothelial cells (CCECs) due to the difficulty of isolating primary cells. We made many attempts to isolate these cells and unfortunately failed. In 1 study conducted in 2009, only 190 differentially expressed genes out of 22,000 possible genes (<0.9%) between CCECs and AECs were detected on an array, indicating that the transcript levels were highly similar between these 2 cell types. 40 In addition, CBS and CSE share the same substrates, and no inhibitor specific to CBS has been detected. 41 Thus, H2S generated by CBS and that produced by CSE cannot be differentiated in assessment of H2S production using homogenates. Consequently, we evaluated H2S generated by CBS and CSE together. Accordingly, H2S produced via the CAT/3-MST and DAO/3-MST pathways was assessed as another mechanism.

CONCLUSIONS

All H2S-generating enzymes are expressed in rat penile tissues. Endogenous H2S production was significantly decreased in the diabetic ED rats’ penile tissues due to downregulated expression of the CAT/3-MST and DAO/3-MST pathways in CCSMCs and low activities of CBS and CSE in CCSMCs and endothelial cells. Based on our results, we hypothesize that downregulation of the CAT/3-MST and DAO/3-MST pathways and decreased activities of CBS and CSE may be related to the development of diabetic ED.

Corresponding Author: Jun Yang, MD, PhD, Department of Urology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei, China. Tel: (86) 027-83663460; Fax: (86) 027-83663460; E-mail: yjun1985win@163.com

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STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Ji-Hong Liu; Chun-Ping Yin
(b) Acquisition of Data
Yan Zhang; Jun Yang
(c) Analysis and Interpretation of Data
Tao Wang; Shao-Gang Wang; Zang-Qun Ye

Category 2

(a) Drafting the Article
Yan Zhang; Jun Yang; Shao-Gang Wang
(b) Revising It for Intellectual Content
Tao Wang; Ji-Hong Liu; Chun-Ping Yin; Zang-Qun Ye

Category 3

(a) Final Approval of the Completed Article
Yani Zhang; Jun Yang; Tao Wang; Shao-Gang Wang; Ji-Hong Liu; Chun-Ping Yin; Zang-Qun Ye

REFERENCES


6. Huang CW, Moore PK. H2S synthesizing enzymes: biochemistry and molecular aspects. Handb Exp Pharmacol 2015; 230:


