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Hydrogen Sulfide (H_2S) and Reactive Oxygen Species (ROS) Scavengers Have a Protective Effect on Carbachol-induced Contractions That are Impaired by High Glucose in Detrusor Smooth Muscle

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What's known on the subject? and What does the study add?

One of the most frequent diabetic complications is urinary bladder dysfunction, which is associated with increased bladder capacity and impaired smooth muscle contractions. Smooth muscle tissues isolated from diabetic rats have altered contractile responses. However, the mechanisms responsible for altered smooth muscle contractility remain poorly understood. Increased production of reactive oxygen species (ROS) plays a role in bladder disorders, and H_2S has a cytoprotective effect that might have a scavenging effect on ROS. It is important to determine the effect of H_2S on impaired detrusor contractility caused by ROSin order to develop new treatment principles.

Abstract

Objective: Urinary bladder dysfunction, that is one of the most common diabetic complications, is associated with bladder overactivity, increased bladder capacity and also impaired bladder smooth muscle contractions. The involvement of hydrogen sulfide (H_2S) in pathological disorders such as diabetes mellitus has been suggested. NaHS-treatment can distinctly reduce high glucose-induce cytotoxicity and oxidative stress. Reactive oxygen species are produced in increased concentrations in diabetes and may cause tissue damage, thus impaired smooth muscle function. The aim of the study was to investigate the role of H_2S and reactive oxygen scavenger (ROS) on carbachol-induced detrusor smooth muscle contractions under high glucose conditions.

Materials and Methods: Cumulative (10 nM-30 µM) carbachol contraction responses were obtained in bladder detrusor smooth muscle strips isolated from male New Zealand albino rabbits bladder in control group and in high glucose conditions (30 min incubation in Krebs' Henseleit solution with high glucose). Responses were repeated in the presence of sodium hydrosulfide (NaHS), catalase, superoxide dismutase (SOD) and their combinations. Contractions were expressed as a percentage of 80 mM K+ response and $p< 0.05$ was accepted as statistically significant.

Results: Cumulative contractile responses were elicited with carbachol in control group and these responses were significantly increased in the presence of high glucose. Increased carbachol contractile responses in high glucose were significantly reduced in the presence of catalase, SOD and NaHS.

Conclusion: Depending on these results we may propose that H_2S donors and ROS scavengers have probable benefits in treating diabetic complications such as urinary bladder dysfunction.

Keywords: Basic science, bladder, carbachol, high glucose, hydrogen sulfide, reactive oxygen scavenger

Introduction

Urinary bladder dysfunction, which is one of the most common diabetic complications, is associated with bladder overactivity, increased bladder capacity, and impaired bladder smooth muscle contraction. The prevalence of dysfunction is between 43% and 87%. It is not life threatening but considerably affects life quality (1). Evaluation of bladder smooth muscle

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contractility is important for understanding the mechanisms underlying diabetic dysfunction. Alterations in contractile responses have been reported in smooth muscle tissues isolated from diabetic models. Increased carbachol-induced contraction was observed in bladder smooth muscle in streptozotocin (STZ)-induced diabetic rats (2). Pretreatment of bladders under high glucose (HG) conditions enhanced carbachol-induced contraction in control animals (3). Nobe et al. (4) showed that glucose-dependent enhancement of contraction in the diabetic bladder is involved in the activation of the Rho kinase and calcium-independent PKC pathways. The increased vascular smooth muscle contraction, which was enhanced under HG conditions, was also reported in a type II diabetic mouse model (5,6). However, the mechanisms responsible for altered smooth muscle contractility remain poorly understood.

The involvement of hydrogen sulfide (H_2S) in pathological disorders such as diabetes mellitus has been suggested although its physiological role is still not known. Increased formation of H_2S and expression of endogenous H_2S-synthesizing enzymes, cystathionine γ-lyase (CSE) and cystathionine b-synthase (CBS), have been demonstrated in the liver and pancreas of STZ-induced diabetic rats (7). Inhibition of CSE, a synthase of endogenous H_2S, promotes endothelial cell dysfunction induced by hyperglycemia (8) and reduces H_2S levels in STZ-induced diabetic rats (9). In diabetic mice, treatment with H_2S can restore nitric oxide efficacy and decrease oxidative stress in the mouse aorta (10). However, possible accompanying changes in the functional effects of H_2S have not been elucidated.

The cytoprotective effect of H_2S may also be attributed to its scavenging effect on reactive oxygen species (ROS). ROS are produced in increased concentrations in pathological conditions such as cardiac ischemia, reperfusion, or sepsis and cause tissue damage. ROS can alter smooth and striated muscle contraction by affecting many intracellular pathways associated with excitation-contraction coupling. It has been shown that carbachol- and potassium-induced contractions are reduced in the presence of hydrogen peroxide in the rat urinary bladder detrusor muscle (11). In our previous study, H_2S reduced carbachol-induced contraction in the permeabilized guinea pig taenia cecum and that intracellular hydrogen peroxide formation and calcium storage mitochondria are responsible for this response (12). It is also known that superoxide anions reduce the release of calcium by preventing the opening of the calcium channels of the sarcoplastic reticulum in the myocardium (13).

The aim of this study was to investigate the role of H_2S and ROS scavengers in alterations of carbachol-induced detrusor smooth muscle contraction under HG conditions.

Materials and Methods

The study was approved by the Hacettepe University Animal Ethics Committee (no: 2023/06-06). Male New Zealand albino rabbits (4-6 months old) were used in this study.

Tissue Preparation

Rabbits were euthanized under high-dose anesthesia (Ketamine/Xylazine, 50/5 mg/kg, i.p.) and their urinary bladders were isolated. Bladder strips were isolated and mounted in 5 mL organ baths containing Krebs' Henseleit solution under a resting tension of 800 mg. Tissues were equilibrated for 1 h and washed with Krebs' Henseleit solution every 15 min before each experimental procedure. Isometric changes in tension were recorded using an isometric force transducer (MP 150-Transducer Data Acquisition System; BIOPAC Systems).

Experimental Protocol

Sodium hydrogen sulfide (NaHS) is used as an H_2S donor, and its aqueous solution is introduced directly into the organ bath by an automated pipette. NaHS dissociates to Na^+ and HS^- in aqueous solution and then HS^- associates with H^+ to form H_2S (Hosoki et al., 1997).

At the beginning of each experiment, KCl (80 mM)-induced contractions were elicited in bladder strips. After a 30-min washout period, cumulative (10 nM-30 μM) carbachol-induced contraction responses were obtained in bladder strips in the control group and under HG conditions. The HG condition means 30 min incubation of bladder strips in Krebs' Henseleit solution with 4.7; $MgSO_4$, 1.2; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; glucose, 11.6 and this was gassed with 95% O_2-5% CO_2 at 37°C and pH 7.4. Krebs' Henseleit solution with HG content contains 44 mM glucose. Cumulative carbachol-induced contraction responses were elicited in the presence of ROS scavengers catalase (1.000 U/mL), superoxide dismutase (SOD; 150 U/mL), H_2S donor sodium hydrosulfide (NaHS, 300 μM), H_2S-synthesizing enzyme inhibitors propargylglycine (PAG; 300 mM) and aminooxyacetic acid (AOAA; 1 mM) in control and HG conditions.

Drugs and Solutions

The drugs used were carbamylcholine chloride (carbachol), catalase, SOD, NaHS, PAG, and AOAA from Sigma (St. Louis, Missouri). All drugs and solutions were prepared by using distilled water.

Statistical Analysis

Contractions are expressed as a percentage of KCl (80 mM)-induced contraction. Data are represented as mean ± standard error of the mean. Statistical analysis was performed by ANOVA/Newman-Keuls and Student's t-test using GraphPad Prism9 software. $P<0.05$ was accepted as statistically significant.

Results

Effect of High Glucose in Bladder Cumulative Carbachol Contraction

Cumulative contractile responses were elicited with carbachol (10 nM-30 μM) in the control group. Bladder strips were incubated with HG (Krebs' Henseleit solution with 44 mM glucose). The contraction responses were significantly increased under HG conditions compared with the control group (Figure 1).

Effects of ROS Scavengers Catalase and SOD on Cumulative Carbachol Contraction

Increased carbachol contractile responses under HG conditions were significantly reduced in the presence of hydrogen peroxide (H_2O_2) scavenger catalase (1000 U/mL) and superoxide (O_2^-) scavenger SOD (150 U/mL) (Figure 2). There was no difference in the control group between the absence and presence of catalase or SOD (Table 1).

Effects of H_2S donor NaHS on Cumulative Carbachol Contraction

Cumulative carbachol (10 nM-30 μM) contractile responses were obtained in the presence of H_2S donor NaHS (300 mM) in control and under HG conditions. Increased carbachol contractile responses under HG conditions were significantly reduced in the presence of NaHS. Contractile responses were also significantly decreased in the presence of NaHS in the control group (Figure 3).

Effects of Combination of H_2S donor NaHS and ROS Scavenger Catalase or SOD on Cumulative Carbachol Contraction

To investigate the interaction between H_2S and ROS, bladder strips were incubated with NaHS and catalase or with NaHS and SOD. There was no further inbibition in cumulative carbachol (10 nM-30 μM) contractile responses incubated with the combination of NaHS and catalase or NaHS and SOD compared with incubation with NaHS alone in the control group. In contrast, further inhibition was observed in carbachol contraction responses under HG conditions when bladder strips were incubated with the combination of NaHS and catalase or NaHS and SOD compared with incubation with NaHS alone (Table 1).

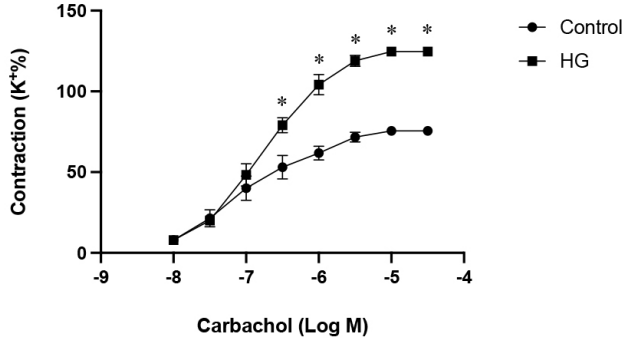

Figure 1. The cumulative contractile response elicited with carbachol (10 nM- 30 μM) in control and HG-incubated bladder detrusor smooth muscle of rabbits (*p<0.05 significant compared to control; n=6)

HG: High glucose

Effects of H_2S-synthesizing Enzyme Inhibitors PAG and AOAA on Cumulative Carbachol Contraction

Increased carbachol contractile responses under HG conditions did not change in the presence of CSE enzyme inhibitör PAG (300 μM) and CBS enzyme inhibitor AOAA (1 mM) (Figure 4).

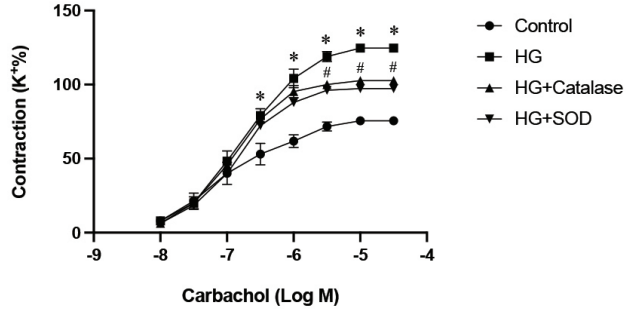

Figure 2. The cumulative contractile response elicited with carbachol (10 nM- 30 μM) in the absence and presence of catalase (1000 U/mL) and SOD (150 U/mL) in control and HG-incubated bladder detrusor smooth muscle of rabbits (*p<0.05 significant compared to control, #p<0.05 significant compared to HG; n=5-6).

HG: High glucose, SOD: Superoxide dismutase

Table 1. Maximum contraction values (E_{max}) obtained with carbachol in the presence of catalase, SOD, NaHS and their combinations in the control and HG-incubated bladder detrusor smooth muscle

Group		E_{max}	n
Control		75.53±2.58	6
HG		124.65±2.36*	6
Catalase	Control	70.88±3.84	5
	HG	102.71±2.71#	5
SOD	Control	72.59±0.80	5
	HG	97.28±0.84#	6
NaHS	Control	64.03±1.72*	5
	HG	94.57±0.97#	5
NaHS + Catalase	Control	66.10±1.18	6
	HG	84.57±0.97#	5
NaHS + SOD	Control	65.47±1.75	5
	HG	85.51±1.26#	5

*: p<0.05 compared to control, #: p<0.05 significant compared to HG, HG: High glucose, NaHS: Sodium hydrogen sulfide, SOD: Superoxide dismutase, max: Maximum

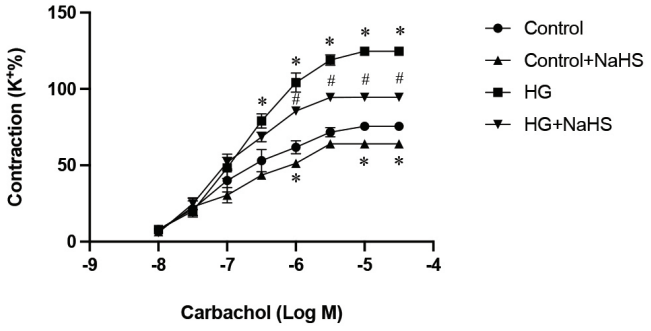

Figure 3. The cumulative contractile response elicited with carbachol (10 nM- 30 µM) in the absence and presence of NaHS (300 µM) in control and HG-incubated bladder detrusor smooth muscle of rabbits (*p<0.05 compared to control, #p<0.05 significant compared to HG; n=5-6)

HG: High glucose, NaHS: Sodium hydrogen sulfide

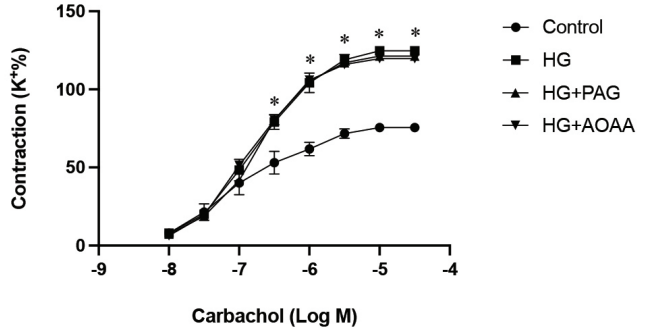

Figure 4. The cumulative contractile response elicited with carbachol (10 nM- 30 µM) in the absence and presence of PAG (300 mM) and AOAA (1 mM) in control and HG-incubated bladder detrusor smooth muscle of rabbits (*p<0.05 compared to control; n=5-6)

PAG: Propargylgylicine, HG: High glucose, AOAA: Aminooxyacetic acid

Discussion

Many neurological, cardiovascular, urological, gastrointestinal, and biochemical complications develop in patients with diabetes due to increased glucose. Bladder dysfunction is one of the most common diabetic complications associated with bladder overactivity, increased bladder capacity, and impaired smooth muscle contractile function. Investigating the mechanism of impaired bladder smooth muscle contractility is important for understanding the underlying mechanisms of diabetes complications. Moreover, bladder dysfunction problems, especially those more common in women, lead to social problems as well. Our aim in this study was to elucidate the effect of ROS scavengers and H_2S on impaired contractile functions under HG conditions.

Changes in contractile responses in bladder smooth muscle experimentally induced or incubated with HG have been reported (2-4). In our study, cumulative carbachol contractile responses were significantly increased in the HG group compared with the control group. An increase in carbachol-induced contractile responses was demonstrated in bladder strips isolated from STZ-induced diabetic rats and in tissues pre-treated with HG (2,3). Many mechanisms are believed to be responsible for the impaired contractile response due to HG. The consequence of hyperglycemic stimulation is the increase of ROS, thus initiating oxidative stress, which causes bladder smooth muscle damage, resulting in impaired bladder function (14-16). Growing evidence has shown that high-glucose-related oxidative stress has an essential role in the remodeling of smooth muscle function that eventually results in the decompensation of the detrusor muscle (17-19). The complications of diabetes are thought to be the result of oxidative stress associated with HG in several tissues (20) including the detrusor smooth muscle (21). It has been reported that repeated stimulation of rabbit bladder strips leads to increased lipid peroxidation and impaired smooth muscle contractility in the ischemic and hypoxic media as well as in the normal physiological media (22).

Samples from rats with STZ-induced type 1 diabetes showed that genes involved in the production or enhancement of ROS and oxidative pathways are upregulated in the bladder of these rats, whereas antioxidative enzymes are downregulated (17-20). Xue et al. (23) 2021 showed that the viability of bladder smooth muscle cells significantly decreased and apoptotic cells increased after HG treatment; at the same time, the SOD level decreased and MDA increased. SOD is an important antioxidant enzyme, and its level decreases, suggesting a decline in antioxidant capacity. MDA is a lipid oxidative damage marker, and its increased level indicates a higher level of oxidative stress (23). In this study, we investigated the effects of ROS scavengers, O^{2-} radical scavenger SOD and H_2O_2 scavenger catalase, on increased carbachol contractile responses under HG. In the present study, we observed that contractile response under HG conditions was decreased in the presence of catalase and SOD. There was no difference in the control group in the presence of catalase and SOD. Consistent with previous studies, our results indicate that HG causes impaired contractile responses in the detrusor smooth muscle through oxidative stress.

Exogenous H_2S significantly prevented cell death, decreased the generation of apoptotic markers, and suppressed mitochondrial ROS production in rat aortic endothelial cells under HG conditions (24). NaHS treatment can distinctly reduce HG-induced cytotoxicity, apoptosis, oxidative stress, and inflammation in HUVECs (25). In diabetic mice, treatment with H_2S can restore nitric oxide efficacy and decrease oxidative stress in the mouse aorta (10). H_2S may act as a cytoprotective

hormone in mouse islets and in MIN6 cells exposed to HG, fatty acids, or a mixture of cytotoxic cytokines (26,27). The effects of H_2S on increased carbachol contractile responses under HG conditions were also investigated. According to our results, increased carbachol contractile responses were significantly reduced under HG conditions in the presence of NaHS. Contractile responses were also significantly decreased in the control group in the presence of NaHS. Inhibition was seen in 14% (the control group) and 24% (HG group) ratios. In parallel with previous studies, our results suggest that H_2S reduces the oxidative stress caused by HG and, as a result, improves the impaired contractile responses. Moreover, when the combined effects of H_2S and ROS scavengers were examined under HG conditions, after incubation of NaHS and catalase or NaHS and SOD together, a further reduction 32% and 31% in carbachol contractile responses were obtained, respectively. According to studies examining the possible interaction of H_2S and ROS, Muzaffar et al. (28) showed that H_2S suppressed O^{2-} production in pulmonary artery endothelial cells. In another study, NaHS infusion decreased O^{2-} production in hypertensive rats (29).

H_2S-synthesizing enzyme inhibitors PAG and AOAA were examined to support the regulating effect of H_2S on deteriorated contractile responses in bladder smooth muscle under HG conditions. Increased carbachol contractile responses under HG were not changed in the presence of CSE enzyme inhibitör PAG and the CBS enzyme inhibitor AOAA.

Study Limitations

The fact that our study is an animal study is an important limitation. It is difficult to mimic hyperglycemia in animal tissues. Studies on the effects of H_2S and ROS on humans should be conducted to strengthen these findings.

Conclusion

The study identified alterations in contractile responses in bladder smooth muscle under HG conditions. Cumulative carbachol-induced contractile responses were significantly increased in HG-incubated bladder detrusor muscle. These increased contractile responses decreased in the presence of catalase, SOD, and NaHS. Therefore, we can suggest that agonist-induced contractile functions in diabetes are related to H_2S and ROS such as H_2O_2 and O_2^-. In conclusion, these results may become a valuable source for assessing the probable benefits of H_2S donors and ROS scavengers in treating diabetic complications such as urinary bladder dysfunction.

Ethics

Ethics Committee Approval: The study was approved by the Hacettepe University Animal Ethics Committee (no: 2023/06-06).

Informed Consent: Not necessary.

Authorship Contributions

Concept: M.D., N.T.D.K., Design: M.D., N.T.D.K., Data Collection or Processing: M.D., Analysis or Interpretation: M.D., N.T.D.K., Literature Search: M.D., Writing: M.D., N.T.D.K.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declare that they have no relevant financial.

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