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A N I N T E R N A T I O N A L J O U R N A L

Decreased mRNA Expression of Cystathionine Gamma lyase and H2S Concentration in Gastric Mucosal Tissue in Diabetic Rats

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ABSTRACT

Background and aim: It is well established that the rate of gastric lesions increases in diabetic rats. Recently, the protective effect of hydrogen sulfide (H_2S) in gastric mucosa has been proven. This study aimed to determine the release of H_2S and mRNA expression of cystathionine gamma lyase (CSE) in gastric mucosa in alloxan-diabetic rats in response to distention-induced gastric acid secretion.
Twenty-four rats were randomly assigned to 4 groups (6 in each). They were the normal-control, distention-control, diabetic-control, and distention-diabetic groups. Under anesthesia, animals underwent a tracheotomy and midline laparotomy. To washout the gastric contents, a catheter was inserted in the stomach through the duodenum. To determine the effect of distention-induced gastric acid secretion on H_2S release and mRNA expression of CSE, the stomachs were distended by normal saline. At the end of experiments, animals were sacrificed and the gastric mucosa was collected to determine H_2S concentration and to quantify mRNA expression of CSE by quantitative real-time PCR.
Mucosal release of H_2S and mRNA expression of CSE significantly increased in response to stimulated gastric acid secretion in normal rats ($P<0.01$), while the increases in diabetic rats were not significant. Basal release of H_2S and mRNA expression of CSE in gastric mucosa were significantly in diabetic rats lower than normal rats.
On the basis of the results, we conclude that the decreased release of H_2S in response to basal and stimulated gastric acid output in alloxan-diabetic rats compared to normal rats is largely due to downregulation of mRNA expression of CSE.

Key words: gastric acid secretion; cystathionine gamma lyase; hydrogen sulfide; alloxan-diabetic rats

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INTRODUCTION

In mammals, hydrogen sulfide (H_2S) is naturally produced by enzymatic and non-enzymatic pathways [1]. It has been shown that cystathionine gamma lyase (CSE) is the main enzyme responsible for H_2S synthesis in the gastric mucosa in rats [2]. H_2S has been shown to inhibit gastric acid release [3], stimulate mucus and bicarbonate secretion[4, 5], and increase gastric mucosal blood flow (GMBF)[6].
Recently, it has been shown that stimulated gastric acid secretion and mucosal acidification increase mucosal release of H_2S in the rat stomach [2, 7].
It has been shown that the increased susceptibility of gastric mucosal tissue to irritants in diabetic animals[8, 9] is due to different alterations in gastric mucosal tissue such as impairment of GMBF, decreased release of calcitonin-gene related peptide (CGRP) from CGRP neurons[9], impairment of the antioxidant system [10], and suppression of basic fibroblast growth factor [11].CGRP mediates the hyperemic response following the back-diffusion of gastric acid, aimed at maintaining the integrity of the gastric mucosal barrier [12, 13].
As far as we know, there is no report about the effect of stimulated gastric acid secretion on gastric H_2S release in diabetic rats. Therefore, the present study aimed to determine the gastric release of H_2S and mRNA expression of CSE in diabetic rats in response to distention-induced gastric acid secretion.

MATERIALS AND METHODS

Animals

Male Wistar rats (150-200 g) were purchased from the animal house of Ahvaz Jundishapur University of Medical Sciences. The animals were fed conventional diet and had free access to tap water. They were maintained under standard conditions of humidity, temperature (22±2°C) and 12-h light/dark cycle. The animals were deprived of food but not water overnight before intervention. All experiments were carried out in accordance with the regulations set by Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (APRC-94-16).

Animal Grouping and Experimental Procedures

Twenty-four rats were randomly assigned to 4 groups (6 in each). The animals were anesthetized with 60 mg/kg ketamine and 15 mg/kg xylazine, i.p., and subjected to midline laparotomy. The depth of anesthesia was checked throughout the experiment by the pedal withdrawal (toe pinch) reflex every 30 min. If the pedal withdrawal reflex was observed, a supplemental dose of ketamine+xylazine (1/3 of initial dose) was administered to maintain adequate anesthesia. Animal body temperature was measured with a rectal thermometer and maintained at 37°C using a homeothermic blanket control system (Harvard, UK). At the beginning of each experiment, the lumen of the stomach was gently rinsed with isotonic saline (37°C, pH7) until gastric washout was clear.
Thirty minutes after surgical operation, 1 ml of isotonic saline (pH 7 and 37°C) was instilled into the stomach in control groups (normal and diabetic) through a duodenal catheter. To evaluate the effect of gastric distention on mucosal release of H_2S and mRNA expression of CSE in gastric mucosa in normal and diabetic rats, the stomach was distended by instilling isotonic saline (1.5 ml/100 g of body weight, pH 7 and 37°C). Ninety minutes after beginning the experiment, animals were sacrificed by an overdose of anesthetics; gastric contents were drained for measuring the pH using a digital pH meter (isTEK, Inc. South Korea). The stomachs were then opened along the greater curvature, rinsed with physiological saline and pinned out in ice-cold

saline. For measuring the mRNA expression of CSE by quantitative real-time PCR and determining H_2S release in the gastric mucosa by an ELISA kit, two samples of gastric mucosal tissue were quickly snap-frozen and stored in liquid nitrogen.

Induction of diabetes

To induce diabetes, overnight fasted rats were given a single intraperitoneal injection of 175 mg/kgalloxan monohydrate. After 72 h, blood glucose was checked with a glucometer (Elegance, Germany). Rats with fasting blood glucose higher than 250 mg/dl were considered diabetic. Two weeks later, animals with fasting blood sugar higher than 250 mg/dl were considered gastroparetic[4, 14].

Quantitative real-time PCR

The mRNA levels of CSE, and the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured by quantitative real-time PCR (qRT-PCR) using a LightCycler® 480 System (Roche Diagnostics). The specific primers (Bioneer, South Korea) for measurement of CSE and GAPDH were used, and the lengths for amplified products were as follows:

GAPDH (forward primer:5´-TGCTGGTGCTGAGTATGTCGTG-3 and reverse primer:5´- CGGAGATGATGACCCTTTTGG-3´, 101 bp) and CSE (forward primer:5´- TGTTGTCATGGGCTTAGTG-3´and reverse primer:5´-CCATCCCATTCCTGAAGTG -3, 167 bp). All PCR amplifications were performed in duplicate and in final volume of 20 µl containing 2 µlof cDNA, 0.8 µl of specific primers, 10 µl of SYBR green master mix [TAKARA SYBR® Premix Ex Taq ™ II (TliRNaseH Plus), Bulk] and 6.4 µl of ddH2O, using the following protocol: pre-incubation at 95°C for 2 min to activate DNA Taq polymerase and 40 two-step cycles with denaturation at 95°C for 20 s, and annealing/extension at 60°C for 1 min. In addition, the no-template negative control (H2O) was routinely run in every PCR. The melting curve was examined at the end of amplification process to ensure the specificity of PCR products. Expression level of CSE was normalized against GAPDH expression (internal calibrator for equal RNA template loading and normalization). To determine the relative quantification of gene expression, the comparative threshold cycle (Ct) method with arithmetic formulae (2−QQCt) was used.

Determination of mucosal H2S levels

To determine the effect of distention-induced gastric acid secretion on H2S release, a commercial ELISA (enzyme-linked immunosorbent assay) Kit (ABIN771902, antibodies-online, USA) was used.

Statistical analysis

Data are shown as mean ± SEM. Statistical analysis was performed by two-way ANOVA followed by Tukey’s post hoc test. Significance was set at $P<0.05$.

RESULTS

Effect of Distention-Induced Gastric Acid Secretion on Mucosal Release of Hydrogen Sulfide in Normal and Alloxan-Induced Diabetic Rats.

As illustrated in Figure 1, the mucosal release of H2S significantly increased in response to stimulated gastric acid secretion in normal rats ($P<0.01$) while the increase in diabetic rats was not significant. Figure 1 also shows that basal and acid-induced release of H2S in the stomach of diabetic rats was significantly lower than in normal rats ($P<0.01$ in both cases).

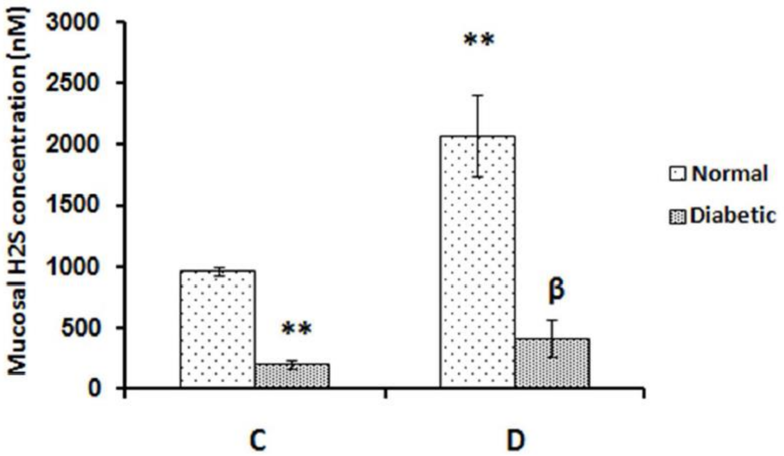

Figure1- Effect of distention-induced gastric acid secretion on mucosal release of H2S in the rat stomach. C: control groups (normal and diabetic rats) and D: distention groups [the acid output was stimulated by gastric distention (normal saline, 1.5 ml/100 g body weight, pH7 and 37°C)]. Analysis of ELISA results showed that the increased mucosal release of H2S in response to distention-induced gastric acid secretion in normal rats was significantly higher than in diabetic rats. **P<0.01 versus normal control group; βP<0.01 versus normal distention group. Data are expressed as mean±SEM.

Effect of Distention-Induced Gastric Acid Secretion on mRna Expression of Cse in Normal and Alloxan-Induced Diabetic Rats

As illustrated in Figure 2, mucosal mRNA expression of CSE significantly increased in response to stimulated gastric acid secretion in normal rats (P<0.01), while the increase in diabetic rats was not significant. Analysis of qRT-PCR results also showed that basal level and acid-induced expression of CSE mRNA in the gastric mucosa was significantly lower in diabetic rats than normal rats (P<0.01 in both cases).

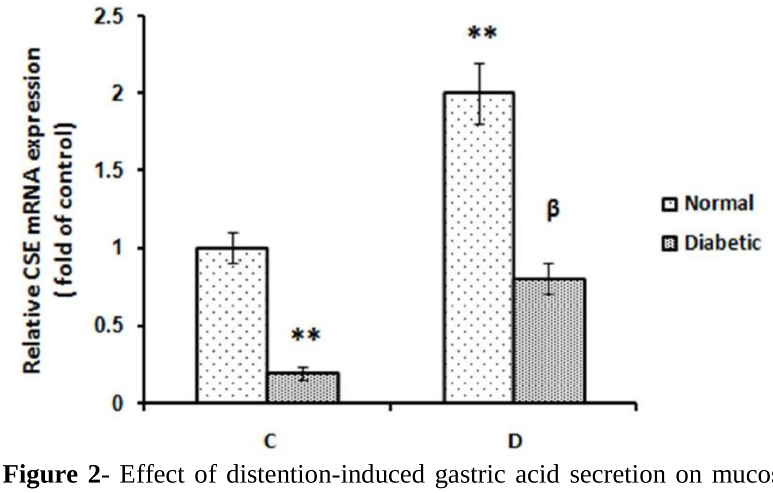

Figure 2- Effect of distention-induced gastric acid secretion on mucosal expression of CSE in the rat stomach. Analysis of qRT-PCR results showed that the increased expression of CSE in response to distention-induced gastric acid secretion in normal rats was significantly higher than in diabetic rats. **P<0.01 versus normal control group; βP<0.01 versus normal distention group. Data are expressed as mean±SEM.

Effect of Gastric Distention on pH of Gastric Contents in Normal and Alloxan-Induced Diabetic Rats

As indicated in Figure 3, gastric distention significantly decreased the pH of gastric contents in normal and diabetic rats (P<0.05 and P<0.05). Figure 3 also shows that the pH of gastric contents in states of control and distention was significantly lower in normal rats than diabetic rats (P<0.01 in both cases). As illustrated in Figure 4, there is a direct correlation between the gastric acid output and mucosal H2S release. This correlation in normal rats was more direct than in diabetic rats. As demonstrated in Figure 3, mucosal acid response to gastric distention and basal acid output were significantly lower in diabetic rats than normal rats as evidenced by the pH of gastric contents.

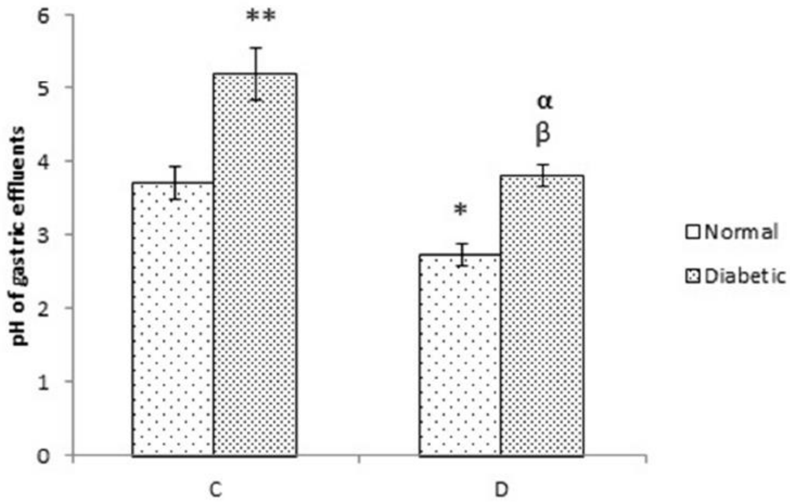

Figure 3- Effect of gastric distention on pH of gastric effluents in normal and alloxan-induced diabetic rats. Gastric distention significantly decreased pH of gastric contents in normal and diabetic rats. pH of gastric contents in states of control and distention in normal rats was significantly lower than in diabetic rats. *P<0.05 and **P<0.01 versus normal control group; βP<0.01 versus normal distention group. αP<0.01 versus diabetic control group. Data are expressed as mean±SEM.

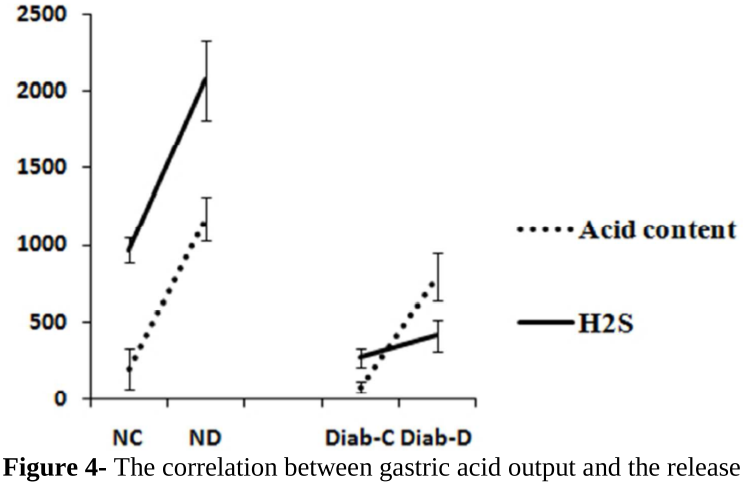

Figure 4- The correlation between gastric acid output and the release of H2S in normal and diabetic rats.

DISCUSSION

The findings of the present study showed that (1) mucosal release of H2S and mRNA expression of CSE increased in response to gastric acid and that (2) the increased levels of H2S and CSE mRNA expression were significantly lower in diabetic rats than normal rats.

The present results showed that mucosal H2S release in the rat stomach increased in response to distention-induced gastric acid secretion in normal rats. Recently, mucosal release of H2S was shown to increase in response to gastric acid and mucosal acidification in normal Wistar rats 2, 7.
Therefore, these findings together demonstrate that any increase in gastric acid output is accompanied by increased mucosal H2S production in the rat stomach.
Consistent with previous findings 2, 7, the present study showed that CSE mRNA expression increased in response to distention-induced gastric distention in normal rats but not significantly in diabetic rats. Therefore, it can be concluded that decreased mucosal release of H2S in response to gastric acid in diabetic rats compared to normal rats is largely due to decreased expression of CSE.
As shown in Figure 3, mucosal acid response to gastric distention and basal acid output were significantly lower in diabetic rats than normal rats as evidenced by the pH of gastric contents.
Reports have shown that gastric acid response to secretagogues changed in diabetes, although some studies showed an increase while others a decrease. One study showed that the rate of basal gastric secretion was similar between normal and diabetic rats, while it was significantly higher in response to pentagastrin and histamine in streptozocin-diabetic rats15. On the other hand, it has been shown that basal and stimulated gastric acid secretion decreases in alloxan- and streptozocin-induced diabetes rats16, 17. Accordingly, our findings showed that basal and stimulated gastric acid output was significantly reduced in alloxan-diabetic rats compared to normal rats. As shown in the current study and previous investigations 2, 7, there is a direct correlation between gastric acid output and H2S release in normal rats, but this correlation was not observed in alloxan-diabetic rats. Increased acid output without enough expected increase in mucosal release of H2S in diabetic rats maybe related to the chemical ablation or decreased activity of CGRP neurons or a decrease in mucosal NO release.
CGRP neurons have been shown to be activated in response to back-diffused gastric acid, mediating the hyperemic response by activating the release of H2S 12, 13. This study did not evaluate the activity of CGRP neurons, but another study showed that the activity of CGRP neurons decreases in the gastric mucosa of diabetic rats 9.Therefore, these results suggest that the decreased level of H2S in diabetic rats is due to a decrease in the activity of CGRP neurons.
Another factor involved in acid-induced H2S release is nitric oxide (NO). Recently, NO was shown to increase the activity and protein expression of CSE in gastric mucosa in response to distention and pentagastrin-induced gastric acid secretion 2. Additionally, NO has been shown to mediate gastric hyperemic response to capsaicin18.One study showed that the luminal release of NO decreased in streptozocin-diabetic rats 19. Therefore, it could be concluded that decreased production of NO leads to a decrease in H2S release in diabetic rats.
As illustrated in Figures 1 and 2, basal and acid-induced mucosal release of H2S and level of CSE mRNA expression were higher in normal rats than diabetic rats. These results demonstrated that under normal conditions, any increase in gastric acid secretion or any decrease in intra-gastric pH is accompanied by increased mucosal H2S production, while in abnormal situations, such as alloxaninduced diabetes as shown by the present data and NSAID-induced gastritis as shown by Fiorucci *et al* 6, increased acid output is not associated with H2S release. Therefore, these findings together imply that increased susceptibility of the gastric mucosa to irritants in diabetes could be due to the decreased expression of CSE and release of hydrogen sulfide.

CONCLUSION

The present findings showed that basal and acid-induced release of H2S decreases in alloxandiabetic rats. Therefore, we conclude that decreased release of H2S in diabetic rats is largely due to downregulation of CSE mRNA expression.

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