

Atrial natriuretic peptide signal pathway upregulated in stomach of streptozotocin-induced diabetic mice

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Abstract

AIM: To investigate atrial natriuretic peptide (ANP) secretion from gastric mucosa and the relationship between the ANP/natriuretic peptide receptor type A (NPR-A) pathway and diabetic gastroparesis.

METHODS: Male imprinting control region (ICR) mice (4 wk old) were divided into two groups: control mice, and streptozotocin-induced diabetic mice. Eight weeks after injection, spontaneous gastric contraction was recorded by using physiography in control and streptozotocin-induced diabetic mice. The ANP-positive cells in gastric mucosa and among dispersed gastric epithelial cells were detected by using immunohistochemistry and flow cytometry, respectively. ANP and natriuretic

peptide receptor type A (NPR-A) gene expression in gastric tissue was observed by using the reverse transcriptase polymerase chain reaction.

RESULTS: The frequency of spontaneous gastric contraction was reduced from 12.9 ± 0.8 cycles/min in the control group to 8.4 ± 0.6 cycles/min in the diabetic mice ($n = 8$, $P < 0.05$). However, the amplitude of contraction was not significantly affected in the diabetic group. The depletion of interstitial cells of Cajal in the gastric muscle layer was observed in the diabetic mice. ANP-positive cells were distributed in the gastric mucosal layer and the density index of ANP-positive cells was increased from 20.9 ± 2.2 cells/field in control mice to 51.8 ± 2.9 cells/field in diabetic mice ($n = 8$, $P < 0.05$). The percentage of ANP-positive cells among the dispersed gastric epithelial cells was increased from $10.0\% \pm 0.9\%$ in the control mice to $41.2\% \pm 1.0\%$ in the diabetic mice ($n = 3$, $P < 0.05$). ANP and NPR-A genes were both expressed in mouse stomach, and the expression was significantly increased in the diabetic mice.

CONCLUSION: These results suggest that the ANP/NPR-A signaling pathway is upregulated in streptozotocin-induced diabetic mice, and contributes to the development of diabetic gastroparesis.

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Key words: Diabetes mellitus; Atrial natriuretic peptide; Gastric mucosa; Gastroparesis

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INTRODUCTION

Gastroparesis is a chronic complication of diabetes, also called delayed gastric emptying, occurs in > 50% of patients with long-standing diabetes^[1]. Symptoms of gastroparesis include heartburn, pain in the upper abdomen, vomiting, nausea, and early feeling of fullness, but the worst effect of gastroparesis is that it can make diabetes worse by making blood glucose control more difficult^[2]. Besides, there are deterioration in glycemic control and incapacitating symptoms such as malnutrition, water and electrolyte imbalance, and aspiration. However, the pathophysiology of diabetic gastropathy and gastroparesis, such as the mechanism of impaired fundic, pyloric relaxation and impaired electrical pacemaking, are still not established^[3,4]. It is generally believed that diabetic gastropathy and gastroparesis may be caused by visceral autonomic neuropathy, hyperglycemia, and degeneration of smooth muscle. Hyperglycemia itself can cause antral hypomotility, gastric dysrhythmia, and delayed gastric emptying in some patients^[5]. Several physiological studies have reported that dysfunction of gastric smooth muscle in diabetes is associated with neural factors and intracellular signaling pathways^[6,7].

Atrial natriuretic peptide (ANP) was isolated from the atrium by de Bold *et al.*^[8] in 1981. From then on, brain natriuretic peptide (BNP), C-type natriuretic peptide (CNP), dendroapsis natriuretic peptide (DNP), micrurus natriuretic peptide (MNP), and ventricular natriuretic peptide (VNP) have been found in succession. Three types of single transmembrane natriuretic peptide receptors (NPRs) for ANP, BNP and CNP have been identified^[9,10], namely, NPR type A (NPR-A), type B (NPR-B), and type C (NPR-C). NPR-A and NPR-B have the membrane-bound particulate guanylate cyclase (pGC), which can catalyze the formation of cGMP from GTP^[8-11]. NPR-A preferentially binds to ANP and BNP, but has a low affinity for CNP. NPR-B has a much higher affinity for CNP than either ANP or BNP^[12]. Besides the heart, ANP is also distributed in other organs, for example, ANP can be secreted by gastric mucosa^[13-15]. It is well known that ANP and other family members exert natriuretic-diuretic effects, vasorelaxation, and other functions including: decreasing blood pressure, and controlling electrolyte homeostasis. Some studies have demonstrated that the natriuretic peptide family plays an inhibitory role in regulating gastrointestinal motility, for example, in the chicken rectum^[3], rat tenia coli^[16] and guinea-pig cecum^[17].

Our previous study also indicated that CNP relaxes gastric circular and longitudinal smooth muscles in human, rat and guinea-pig stomach, and NPRs are distributed in rat gastric smooth muscle layer^[18-20]. Recently, we have also reported that the CNP-induced

relaxation and the production of cGMP of gastric smooth muscle are potentiated in streptozotocin (STZ)-induced diabetic rats. As well as the activity of pGC, the expression of *NPR-B* gene in gastric smooth muscle is upregulated in STZ-induced diabetic rats^[21]. These results suggest that the CNP/(NPR-B)/pGC/cGMP signaling pathway is involved in the pathogenesis of diabetes. Our previous studies have confirmed that ANP-synthesizing cells exist in different regions of the gastric mucosa in rats^[15], therefore, ANP can be considered an endogenous natriuretic peptide of gastric mucosa. However, it is not clear whether the ANP/NPR-A signaling pathway is involved in the pathogenesis of diabetic gastroparesis.

In the present study, we investigated the relationship between the ANP signaling pathway and STZ-induced diabetic gastroparesis to confirm whether ANP contributes to the development of gastroparesis. The present study focused on whether the amount of ANP secretion from gastric mucosa was enhanced and the expression of the *NPR-A* gene in gastric smooth muscle was upregulated in a mouse model of STZ-induced diabetic gastroparesis.

MATERIALS AND METHODS

Drugs

STZ, TRIzol Reagent and chemicals were purchased from Sigma. C-kit antibody, ANP antibody and pronase were purchased from Santa Cruz Biotechnology and Roche. Other chemicals were purchased from Sangon Biological Company.

STZ-induced diabetic mouse model

Male imprinting control region (ICR) mice (4 wk old) were purchased from the Experimental Animal Center of Shanghai Jiaotong University School of Medicine. A total of 80 mice were divided into two groups: control group and STZ-induced diabetic group. STZ-induced diabetes was created as follows: the mice were fasted overnight and intraperitoneally administered STZ solution (Sigma-Aldrich, St. Louis, MO, USA). STZ was diluted in 0.1 mol/L citrate buffer (pH = 4.0) and used at a dose of 200 mg/kg. Control mice were intraperitoneally administered with the same volume of 0.1 mol/L citrate buffer. The glucose concentration of blood was determined with One-touch Apparatus (Johnson & Johnson Medical Company). STZ-induced diabetic mice were confirmed by measuring glucose concentration from tail blood after fasting, and diabetes was defined when the blood glucose level was > 16 mmol/L. All experimental protocols included in this study were approved by the local Animal Care Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the Science and Technology Commission of P.R.C. (STCC Publication No. 2, revised 1988).

Gastric motility in mouse intact stomach

Eight weeks after treatment with STZ, the animals

were euthanized by lethal dose of intraperitoneal pentobarbital sodium (50 mg/kg). The abdomen of each mouse was opened along the midline, and the intact stomach was removed and placed in pre-oxygenated Krebs solution (containing in 118.1 mmol/L NaCl, 4.7 mmol/L KCl, 1.0 mmol/L KH₂PO₄, 1.0 mmol/L MgSO₄, 25.0 mmol/L NaHCO₃, 2.5 mmol/L CaCl₂, and 11.1 mmol/L glucose), which was equilibrated with 95% oxygen and 5% CO₂. The connective tissue was removed and the pylorus was connected to a pressure transducer (Chengdu Equipment Factory, China) with a thin glass tubule, and the gastric cardia was tied with thin string. The stomach was incubated in a 15-mL organ bath filled with Krebs solution and gassed with 95% O₂ and 5% CO₂ at 37°C. The sensitivity of the pressure transducer was adjusted to an appropriate value and we recorded gastric motility by using the SMUP-E biological signal processing system (Chengdu Equipment Factory). The stomach was allowed to incubate for at least 60 min before the experiments were started, and we eliminated error by injecting an equal volume of Krebs solution into the stomach.

Immunohistochemistry

The mice were euthanized by lethal dose of intraperitoneal pentobarbital sodium (50 mg/kg), the stomach was removed and washed with saline, and then fixed in 4% paraformaldehyde (4°C, 24 h). The fixed tissue was washed with running water (room temperature, 2 h), immersed with 95% and 100% alcohol (room temperature, 2 × 2 h), xylene (2 × 20 min) and paraffin (68.5°C, 30/40/50 min). Sections of 6 μm thickness were cut and deparaffinized in xylene (4 × 30 min). The specimens were hydrated in graded concentrations of ethanol, and washed three times in PBS, and incubated in the blocking reagent for 30 min. The samples were incubated with polyclonal antibody against c-Kit protein (sc-5535; Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:100 dilution). c-Kit protein was used as a marker of interstitial cells of Cajal (ICCs)^[22,23] and with polyclonal antibody against ANP (sc-20158; Santa Cruz Biotechnology; 1:100 dilution) for 24 h at 4°C, followed by 3 h incubation in Rho-anti-rabbit and horseradish peroxidase-anti-rabbit second antibody, respectively. The negative control group was omitted by incubating with primary antibody against ANP. The slice was visualized and photographed under a fluorescence microscope (Olympus IX71, Tokyo, Japan).

Flow cytometry analysis of gastric epithelial cell

Eight weeks after injection with STZ, the mice were euthanized by lethal dose of intraperitoneal pentobarbital sodium (50 mg/kg). The stomach was removed and washed with saline. After the stomachs were inverted to make mucosal-side-out stomachs, the stomachs were rinsed with saline, and pronase solution was injected into the stomach. The pronase was diluted into 1 mg/mL with MA solution (0.5 mmol/L NaH₂PO₄, 1 mmol/L Na₂HPO₄, 20 mmol/L NaHCO₃, 80 mmol/L NaCl,

5 mmol/L KCl, 50 mmol/L HEPES, 11 mmol/L glucose, 20 g/L BSA, 2 mmol/L EDTA; pH = 7.4). The stomach sacks were incubated in the oxygenated MA solution for 3 × 30 min, at 37°C equilibrated with 95% oxygen and 5% CO₂, followed by gently stirring for 1 h in MB solution (0.5 mmol/L NaH₂PO₄, 1 mmol/L Na₂HPO₄, 20 mmol/L NaHCO₃, 80 mmol/L NaCl, 5 mmol/L KCl, 50 mmol/L HEPES, 11 mmol/L glucose, 10 g/L BSA, 1 mmol/L CaCl₂, 1.5 mmol/L MgCl₂; pH = 7.4). MB solution that contained the gastric mucosal cells was collected, and filtered through a 200-mesh cellular sieve. The solution sample was centrifuged at 1500 × g for 5 min. The centrifuged sample was re-suspended with MC solution (0.5 mmol/L NaH₂PO₄, 1 mmol/L Na₂HPO₄, 20 mmol/L NaHCO₃, 80 mmol/L NaCl, 5 mmol/L KCl, 50 mmol/L HEPES, 11 mmol/L glucose, 1 mmol/L CaCl₂, 1.5 mmol/L MgCl₂, 1 mmol/L dithiothreitol; pH = 7.4) and centrifuged at 1500 × g for 5 min again. The density of the cell suspension was adjusted to 3 × 10⁶/mL. The cells were fixed with 75% cold alcohol (-20°C, 24 h), and washed with PBS, followed by 0.2% Triton X-100 for 10 min. The antigen was blocked by 10% goat serum diluted in PBS, and incubated with antibody against ANP (sc-20158; Santa Cruz Biotechnology; 1:50 dilution) overnight at 4°C. The cells were stained with FITC-conjugated goat anti-rabbit IgG and examined by flow cytometry (Becton Dickinson). Using Cellquest software, 10⁴ cells were analyzed per sample.

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of ANP, and NPR-A gene expression

The whole gastric tissue was quickly removed from the mouse. Total RNA was isolated from the tissue as recommended by the manufacturer of TRIzol Reagent (Sigma). RNA concentration was determined by absorbance reading at 260/280 nm. Reverse transcription was performed with a volume of 20 μL mixture that contained 11 μL mRNA, and 1 μL oligo dt₁₈, which was incubated at 70°C for 5 min, and 4 μL 5 × reaction buffer, 2 μL dNTP, 1 μL RNase inhibitor, 1 μL M-MLV RT was added to the mixture, followed by incubation at 42°C for 1 h. The enzyme was inactivated by heating at 70°C for 10 min. cDNA samples were used for analyzing specific cDNA of ANP and NPR-A. One microliter of cDNA was added to 19 μL PCR reaction mixture that contained: 7 μL nuclease-free water, 10 μL 2 × reaction buffer, 1 μL sense primer, and 1 μL anti-sense primer. The following conditions were used for PCR amplification: for GAPDH, 95°C for 4 min; 95°C for 30 s; followed by 40 cycles at 52.9°C for 1 min; 72°C for 30 s; 72°C for 7 min; for ANP, 40 cycles at 54.6°C for 30 s; for NPR-A, 60°C for 30 s. RT-PCR was performed on an iCycler™ Thermal Cycler (Bio-Rad, Hercules, CA, USA) using the Access RT-PCR System (Promega, Madison, WI, USA). Specific primers for murine GAPDH, ANP and NPR-A were synthesized by Sangon Biological Company (Shanghai, China). The primer sequences were as follows: GAPDH (sense) 5'TCAACGGCACAGTCAAGG3', GAPDH (antisense) 5'ACCAGTGGATGCAGGGAT3'; ANP

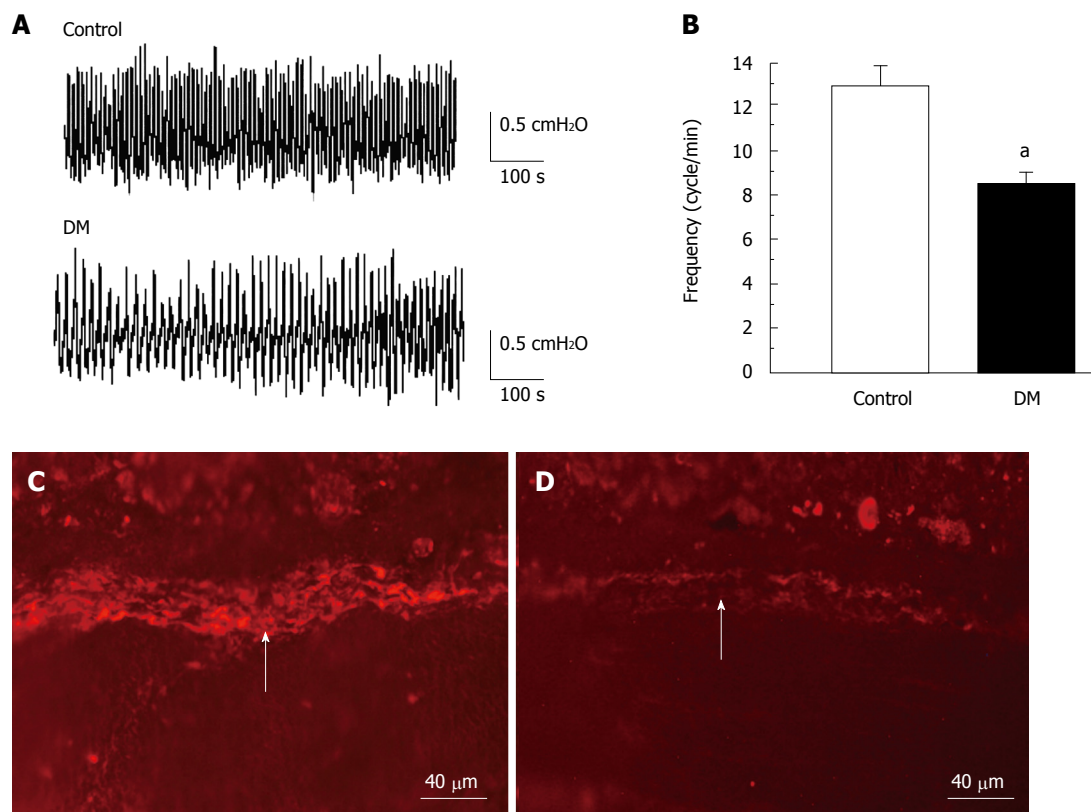


Figure 1 Comparison of gastric contractility and expression of ICCs in normal and STZ-induced diabetic mice. A: Representative traces of spontaneous gastric contraction in control and STZ-induced diabetic mice; B: Summary of amplitudes and frequencies of gastric motility in control and STZ-induced diabetic groups ($n = 8$; $^aP < 0.05$ vs control group); C and D: Expression of c-Kit-positive cells in the muscle layer. Arrow indicates the positive staining area. Expression of c-Kit was more obvious in STZ-induced diabetic mice than in the normal controls. Scale bar = 40 μm .

(sense) 5'TCCTTCTCCATCACCCTG3', ANP (antisense) 5'CCTAGAGCACTGCCGTCT3'; NPRA (sense) 5'AGACGATGGGCAGGATAG3', NPRA (antisense) 5'GGATGTCAGGAGGTGGGT3'. The PCR products were size-fractionated by 1% agarose gel electrophoresis, and visualized under ultraviolet light with 0.5% ethidium bromide staining. GAPDH, ANP and NPR-A cDNAs were quantified by Gel Doc XR System and Quantity One software (Bio-Rad). Gene expression for ANP and NPR-A was normalized to that for GAPDH.

Statistical analysis

The data was expressed as the mean \pm SE. We evaluated differences between the treatment groups using Student's *t* test. Differences were considered to be significant at $P < 0.05$. The density index referred to the number of ANP-positive cell per field of vision.

RESULTS

Change in plasma glucose concentration

We detected changes in blood glucose concentration. Mice were used at 8 wk after injection of STZ. At the time of the study, most STZ-treated mice exhibited hyperglycemia; their average blood glucose concentration was 26.7 ± 1.3 mmol/L ($n = 32$), which was significantly higher than that of the non-diabetic control mice (6.9 ± 0.6 mmol/L, $n = 32$; $P < 0.01$).

Gastric motility and ICC expression in STZ-induced diabetic mice

To determine whether diabetic gastropathy occurred, the amplitude and frequency of spontaneous gastric contraction were observed in normal and STZ-induced diabetic mice. The frequency of spontaneous gastric contraction decreased significantly from 12.9 ± 0.8 cycles/min in the control group to 8.4 ± 0.6 cycles/min in diabetic mice ($n = 8$, $P < 0.05$; Figure 1A and B). However, the amplitude of contraction was not changed in the diabetic group. Spontaneous rhythmic contraction of gastrointestinal smooth muscle is triggered by ICCs, which are also mediators of neuromuscular transmission in the gastrointestinal tract. Depletion of ICCs contributes to dysfunction of gastrointestinal motility in patients and animal models^[24]. ICCs distributed in the gastric smooth muscle layer were observed by immunohistochemistry. c-Kit protein, an ICC marker, was detected in the inter-muscular layer of normal and diabetic mice (Figure 1C and D), but the expression was significantly decreased in the diabetic group ($n = 6$; Figure 1D). The results suggested that the STZ-induced diabetic mice exhibited gastric dysfunction, and the reduced frequency of gastric motility in diabetic mice might have been related to ICC depletion.

ANP secretion from gastric mucosa

Besides the heart, the ANP family is also distributed in other organs, and some studies have found that ANP

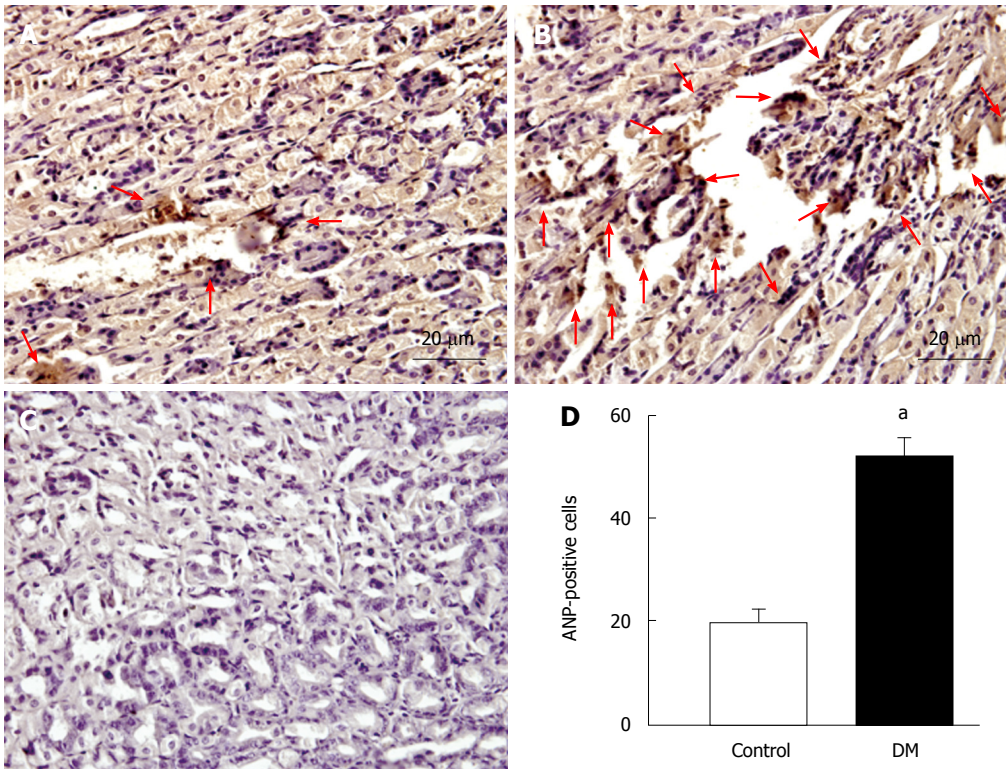


Figure 2 Immunohistochemical analysis of atrial natriuretic peptide (ANP) in mouse gastric tissue. The photographs show ANP expression in gastric mucosa in the control group (A), STZ-induced diabetic group (B), and negative control group (C); The paranuclear cytoplasmic stained cells that display an intense dark brown color were ANP-positive-staining cells in the mucosa. The red arrows indicate ANP-positive cells; B shows that the number of ANP-positive cells increased in the STZ-induced diabetic group; D shows the density index that referred to the number of ANP-positive cells per field of vision ($n = 8$, $^aP < 0.05$ vs control group). Scale bar = 20 μm .

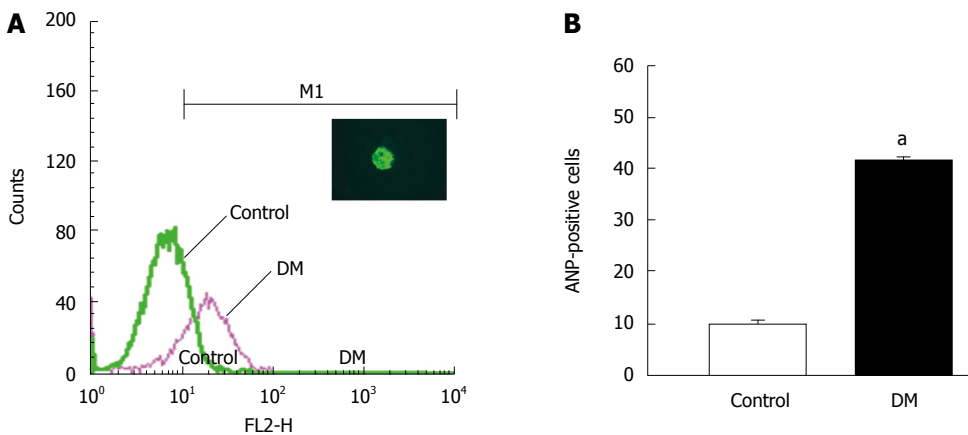


Figure 3 Flow cytometric analysis of ANP-positive cells among gastric mucosal cells. The percentage of ANP-positive cells was determined by flow cytometry with FITC-conjugated goat anti-rabbit IgG. A: Relative fluorescence intensity of gastric mucosal cells in the control and diabetic groups. M1 indicates the proportions of ANP-positive cells that displayed positive FITC labeling. The inserted fluorescent image is a single ANP-positive cell that had a positive fluorescent signal in its cytoplasm ($\times 400$); B: ANP-positive cells among the gastric mucosal cells (10^4 cells per sample) in control and STZ-induced diabetic mice ($n = 3$, $^aP < 0.05$ vs control).

can be secreted from gastric mucosa^[13-15]. To determine whether ANP secretion from gastric mucosa is enhanced in STZ-induced diabetic mice, ANP-positive cells in the gastric mucosa were observed in the control (Figure 2A) and STZ-induced diabetic (Figure 2B) mice. The number of ANP-positive cells was increased significantly in the gastric mucosa of diabetic mice, and the density index was enhanced from 20.9 ± 2.2 cells/field of vision in the controls to 51.8 ± 2.9 cells/field of vision in the diabetic

group ($n = 8$, $P < 0.05$; Figure 2D). We further analyzed the number of ANP-positive cells in gastric epithelial cells by flow cytometry. The relative fluorescence intensity of the diabetic group was higher than that of the control group (Figure 3A). The number of ANP-positive cells was increased significantly in the diabetic group. The percentage of ANP-positive cells among dispersed epithelial cells was enhanced from $10.0\% \pm 0.9\%$ in the control group to $41.2\% \pm 1.0\%$ in the

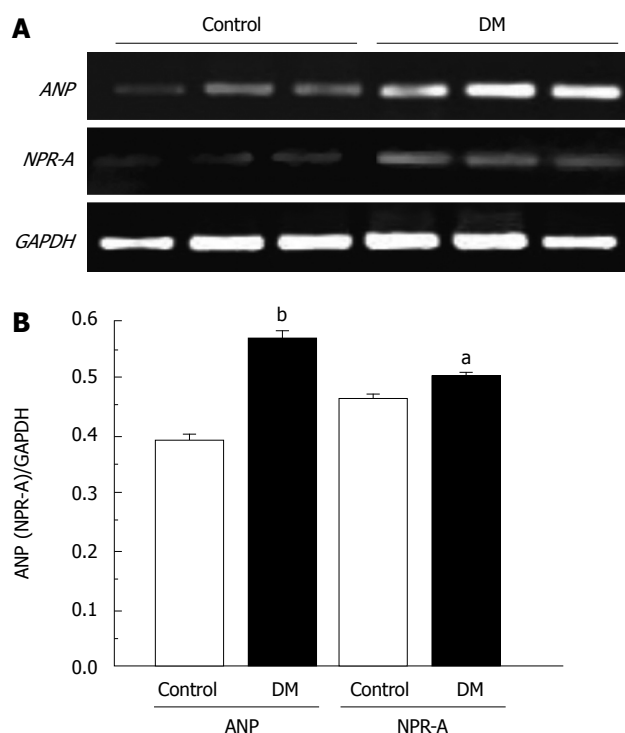


Figure 4 RT-PCR of gastric *ANP* and *NPR-A* genes in control and STZ-induced diabetic mice. A: Results of agarose gel electrophoresis, including *ANP*, *NPR-A* and *GAPDH* genes. The density signal was normalized to that of *GAPDH*. The mean value of the density was obtained from three separate experiments; B: Expression of *ANP* and *NPR-A* genes was increased in STZ-induced diabetic mice ($n = 3$; $^*P < 0.05$, $^bP < 0.01$ vs control).

diabetic group ($n = 3$, $P < 0.05$; Figure 3B). The results suggested that ANP secretion from gastric mucosa was potentiated in the stomach of STZ-induced diabetic mice.

Expression of ANP and NPR-A gene

The number of ANP-positive cells was increased in the STZ-induced diabetic group, therefore, we investigated whether *ANP* and *NPR-A* gene expression was also increased. *ANP* and *NPR-A* gene expression was observed in gastric tissue by RT-PCR. *ANP* and *NPR-A* gene expression was increased in the diabetic group ($n = 3$; Figure 4A). The signal was normalized to that of *GAPDH*. The ratio of ANP/*GAPDH* and NPR-A/*GAPDH* was 0.54 ± 0.02 and 0.5 ± 0.01 and 0.39 ± 0.01 and 0.46 ± 0.02 in the STZ-induced diabetic and control group, respectively ($n = 3$, $P < 0.05$ and $P < 0.01$; Figure 4B).

DISCUSSION

Diabetic gastroparesis is defined as slow gastric emptying in the absence of mechanical obstruction, and constipation is considered to be the most significant clinical manifestation. It occurs in 30%-60% of diabetic patients observed at tertiary referral centers^[25]. Many investigators view these changes as the consequences of irreversible autonomic neuropathy, which affects the

vagus and sympathetic nerves primarily^[2,5]. However, the cause of gastroparesis is multifactorial and also involves enteric neurons, mucosal endocrine cells, smooth muscle cells and ICCs^[4]. The main conclusion drawn from the present study is that the ANP/NPR-A signaling pathway is upregulated and may contribute to the development of gastroparesis in STZ-induced diabetic mice, and then lead to gastric motility dysfunction.

In non-obese diabetic (NOD) mice, a well-established model of human type I diabetes mellitus, gastric emptying of solids is delayed after 6-8 wk of untreated diabetes^[23]. In the present study, we established an STZ-induced diabetic model and gastric motility was observed *in vitro* after 8 wk of untreated diabetes. The frequency of spontaneous contraction in the STZ-induced diabetic group was decreased significantly, however, the amplitude of contraction was not significantly different between these two groups. The results suggest that diabetic gastropathy mostly exhibits slow rhythm and turbulence, however, the amplitude of spontaneous contraction is not affected significantly in the early stage of diabetes. ICCs play a key role in gastric motility, and damage to the ICC networks may contribute to the development of gastropathy and gastroparesis^[26]. In the present study, we confirmed whether the number of ICCs in the gastric smooth muscle layer was changed, by using immunohistochemistry in the STZ-induced diabetic mice. c-Kit expression in the muscle layer was observed in normal and diabetic mice, however, c-Kit expression was decreased in diabetic mice. The results suggest that gastric dysfunction in STZ-induced diabetic mice may be related to ICC depletion.

Atrial myocytes are the main source of ANP, but ANP is also found in other tissues, such as the gastrointestinal tract^[27]. ANP and its receptor NPR-A have been detected in the gastric antrum^[28]. NPR-A has the same membrane-bound guanylate cyclase activity as NPR-B, which catalyzes the formation of cGMP from GTP^[9,10]. Some studies have demonstrated that ANP has an inhibitory effect on the regulation of gastrointestinal motility; for example, in chicken rectum^[16], rat tenia coli^[17] and guinea-pig cecum^[3]. Our previous study also has indicated that NPs relax gastric circular and longitudinal smooth muscles in human, rat and guinea-pig stomach, and NPRs are distributed in the rat gastric smooth muscle layer^[18,19]. Another previous study has indicated that ANP release is augmented in the atrium of STZ-induced diabetic rats^[29]. Recently, we have found that the expression of NPR-B gene is increased and NP-dependent guanylate cyclase/cGMP signaling is upregulated in STZ-induced diabetic rats^[20,21]. ANP is also secreted from the gastric mucosa^[15], therefore, ANP can be considered endogenous to the gastric mucosa. However, the relationship between the ANP/NPR-A signaling pathway and STZ-induced diabetic gastroparesis is not clear. In the present study, we confirmed that the expression of ANP in gastric mucosa was increased significantly in STZ-induced diabetic mice. We demonstrated that the percentage of ANP-positive

cells was also enhanced significantly among the dispersed gastric epithelial cells, and the expression of the *ANP* gene in gastric tissue was upregulated in STZ-induced diabetic mice. These results suggest that ANP secretion from gastric mucosa is increased significantly in STZ-induced diabetes. The amount of ANP secretion is increased in diabetic mice, therefore, we investigated whether expression of NPR-A in the stomach was upregulated in STZ-induced diabetic mice. We demonstrated that expression of the *NPR-A* gene was also increased significantly in gastric tissue of diabetic mice. The results suggest that the ANP/NPR-A signaling pathway is upregulated in the stomach of STZ-induced diabetic mice.

Our previous study has demonstrated that the CNP/NPR-B signaling pathway is upregulated in STZ-induced diabetic rats^[20,21], while the present study indicates that the ANP/NPR-A signaling pathway is also upregulated in STZ-induced diabetic mice. Previous studies have indicated that diabetes may also affect expression of the *ANP* gene; for example, *ANP* gene expression in heart and kidney are increased in STZ-induced diabetic rats^[11,30] and plasma concentration of pro-ANP is increased in comparison with control rats^[31]. Christoffersen *et al.*^[32] also have reported that diabetic mice show an increase in *NPR-B* gene expression in the heart, and have suggested that increased NPR-B signaling affects development of diabetic cardiomyopathy. The NP/NPR signaling pathway has an inhibitory effect on gastrointestinal motility, therefore, upregulation of this signaling pathway may be involved in development of gastroparesis in STZ-induced diabetic mice.

In summary, gastroenteropathy causes considerable morbidity in patients with diabetes mellitus and it has become a major healthcare burden. Current treatments are mainly symptomatic and frequently ineffective. Development of new therapeutic options is hampered because of poor understanding of the underlying pathological mechanisms. Our study demonstrates that the ANP/NPR-A signaling pathway is upregulated. The results suggest that ANP/NPR-A signaling is involved in the development of gastroparesis in STZ-induced diabetic mice.

COMMENTS

Background

Gastroparesis is a gastrointestinal complication of diabetes, which is also called delayed gastric emptying. The mechanism of gastroparesis is not clear, but a recent study has reported that the C-type natriuretic peptide-natriuretic peptide receptor B (CNP/NPR-B) pathway is upregulated in the stomach of diabetic rats. Atrial natriuretic peptide (ANP) has an inhibitory effect on motility of the gastrointestinal tract, but whether secretion of ANP is increased in the stomach of diabetic mice has not been reported.

Research frontiers

NPs are located in several organs besides the heart. Recently, the authors have found that ANP and its receptor (NPR-A) are also present in the mouse stomach, and the CNP/cGMP pathway in diabetic gastroparesis was investigated in STZ-induced diabetic rats. This study was designed to investigate whether ANP secretion is altered in the stomach of STZ-induced diabetic mice.

Innovations and breakthroughs

ANP with an inhibitory effect on gastrointestinal motility is present in the

stomach, and previous studies have focused on the relationship between the CNP/NPR-B pathway and gastric motility. This is believed to be the first study to investigate ANP secretion and NPR-A in the stomach of STZ-induced diabetic mice. The results suggest that ANP secretion and expression of NPR-A mRNA are both increased in the stomach of STZ-induced diabetic mice. This may be involved in stomach motility dysfunction in STZ-induced diabetic mice.

Applications

The dysfunction of stomach motility that occurs in diabetic gastroparesis may be related to ANP secretion and upregulation of NPR-A. This may contribute to the treatment and preventive intervention of diabetic gastroparesis in the future.

Terminology

Gastroparesis also called delayed gastric emptying, and usually occurs in diabetes. Symptoms of gastroparesis include heartburn, pain in the upper abdomen, vomiting, nausea, and early feeling of fullness.

Peer review

This study deals with questions about the mechanisms involved in gastroparesis in diabetes.

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