Alteration of the responses of gastric smooth muscle to endothelin in streptozotocin-induced diabetic rats

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Abstract

Diabetic gastropathy is suggested to be the result of not only an autonomic neuropathy but also to disorder of the spontaneous rhythmic motility of the gastric smooth muscle. Attempts were made to investigate the alteration of the effects of endothelin-1 (ET-1), which is known to enhance the spontaneous activity of gastrointestinal smooth muscle, on gastric activity in streptozotocin (STZ)-induced diabetic rats. STZ-induced diabetic rats were prepared by the injection of Sprague-Dawley (SD) rats with STZ (i.p.). Isometric mechanical responses were recorded in isolated circular smooth muscle strips of the stomach antrum, to measure changes in the rhythmicity of the smooth muscle. ET-1 (10 nM) significantly elevated the resting tension and the frequency of spontaneous contraction, but did not alter the amplitude of the spontaneous oscillatory contractions in normal rats. In diabetic rats, ET-1 elevated the resting tension, and spontaneous contractions were increased in frequency, however they were decreased in amplitude. In normal rats, sarafotoxin S6c (S6c, 10 nM), a selective ETB receptor agonist, elevated the resting tension slightly and increased both the frequency and amplitude of the spontaneous contractions. However, S6c significantly elevated the resting tension alone in STZ-induced diabetic rats. Selective stimulation of endothelin type A (ETₐ) receptors with ET-1, in the presence of a selective antagonist of ETB receptors, produced similar responses in the gastric muscle of both normal and diabetic rats. These results indicate that ET-1 elevates the resting tension and increases the frequency of the spontaneous oscillatory contractions in both normal and STZ-induced diabetic rats, to a similar extent. However, the specific actions on ETB receptors were quite different between the two: the elevating actions on the resting tension were much greater in STZ-diabetic rats than in normal rats. The results suggested the facilitation of ETB receptor signaling in the antrum during the pathogenesis of diabetic gastropathy.

Key words: gastric smooth muscle, mechanical response, endothelin, streptozotocin, diabetes mellitus
Introduction

Diabetic gastropathy refers to a wide range of abnormalities including gastric dysrhythmia and gastroparesis. Diabetic gastroparesis is the result of a systemic and/or enteric neuropathy in the stomach (Koch, 1999). Several physiological studies have demonstrated that spontaneous rhythmic activity of gastric and colonic smooth muscle cells are attenuated in diabetic rats (Xue and Suzuki, 1997; Imaeda et al., 1998; Takano et al., 1998). Interstitial cells of Cajal (ICC) are responsible for the generation of spontaneous electrical activity, such as slow waves, in the stomach (Ward et al., 1994; Huizinga et al., 1995; Sanders, 1996, Dickens et al., 1999, 2001), and a reduction in ICC population is accompanied by gastric dysmotility in diabetic mice (Ördög et al., 2000; Horvath et al., 2005). The inositol triphosphate (IP$_3$) pathway is essential for the generation of gastric slow waves (Suzuki et al., 2000), and the possibility that there is a reduced function of these IP$_3$ pathways in diabetic mice is also considered (Horvath et al., 2005).

Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor which is composed of 21-amino acids (Yanagisawa et al., 1988). The effects of ET-1 on smooth muscle are mediated through at least two distinct subtypes of receptors, namely the endothelin type A (ET$_A$) receptors and the endothelin type B (ET$_B$) receptors (Sakurai et al., 1990). ET-1 acts through the G-protein-IP$_3$ pathway, which increases intracellular free Ca$^{2+}$ by triggering influx from outside of the cells and the release of Ca$^{2+}$ from intracellular stores (Rybaney and Polokoff, 1994).

The following studies have reported the diverse effects of ET-1 on vascular and non-vascular smooth muscle. In several regions of the gastrointestinal tract, ET-1 elicits biphasic effects on smooth muscle, with an initial relaxation followed by a sustained contraction (Lin and Lee, 1990; Allocock et al., 1995; Chakder and Ratten, 1999; Imaeda et al., 2002). ET-1 also has dual effects on slow waves in the rat gastric antrum, through different types of receptors. ET-1 hyperpolarizes the membrane via ET$_A$ receptors, and depolarizes the membrane and increases the frequency of slow waves via ET$_B$ receptors (Imaeda et al., 2004).

In the present study, we investigated the alteration of the effects of ET-1 during the development of diabetic mellitus. We also investigated the relationship between gastropathy and the disorder of the function of ET receptors in streptozotocin-induced diabetic rats. The results indicate that diabetic gastropathy is associated with a disorder of the function of ET$_B$ receptors.

Methods

Animals and experimental design

Male Sprague-Dawley (SD) rats (6 weeks old and weighing 250–400 g) received a single intraperitoneal injection of streptozotocin (STZ, 80 mg/Kg body weight) dissolved in a citrate buffer. Age-matched control rats were injected with citrate buffer alone. Food and water were given ad libitum. At 6 weeks following the injection of STZ or buffer, the blood glucose level was measured using a coulometric glucose sensor (Freestyle, Nipro, Osaka, Japan), taking blood from the tail artery by incising with a sharp knife. This study was approved by the Animal Ethics Committee of the Nagoya City University Graduate School of Medical Sciences.
Tissue preparation

Both normal rats and STZ-induced diabetic rats were anaesthetized with Cevo fren and then decapitated 6 weeks following the injection of either STZ or buffer. The stomach was quickly excised and placed in Krebs solution at room temperature. The stomach was cut open in the longitudinal direction along the lesser curvature and pinned flat with the mucosal side up. The mucosa was gently removed with micro-scissors. Full thickness strips (1–2 mm wide and 8–10 mm long) were cut from the area of the antrum just to the oral side of the thickened prepyloric area. A strip usually included a few bundles of circular smooth muscle.

Mechanical responses

A strip of antrum was tied at each end with fine silk thread and mounted in an organ bath (capacity 1.5 ml), which was perfused with warmed (35°C) Krebs solution at a rate of 2–3 ml min⁻¹. The solution was bubbled with 95% O₂–5% CO₂ to maintain the pH in the range of 7.3–7.4. The ionic composition of the Krebs solution was (mM): NaCl, 118.4; NaHCO₃, 25.0; NaH₂PO₄, 1.13; CaCl₂, 2.4; KCl, 4.7; MgCl₂, 1.3; glucose, 11.1. One end of the strip was fixed at the bottom of the bath, and the other was attached to a force transducer (TB-612T, Nihon Kohden, Tokyo, Japan) by means of silk thread for the measurement of the isometric tension. An initial passive tension of 1 g was applied to each strip and was incubated for at least 1 h before the experiments were started. The majority of preparations started to show spontaneous rhythmical contractions after about 30 minutes following the application of the initial passive tension. When the spontaneous activity of the preparation became stable, we commenced measurement.

Drugs

Drugs used were atropine, BQ123, BQ788, endothelin-1 (ET-1), guanethidine, streptozotocin (STZ), sarafotoxin S6c (S6c), tetrodotoxin (TTX) (all from Sigma, St. Louis, MO, U.S.A.). Individual drugs were dissolved in distilled water or dimethyl sulfoxide (DMSO; Sigma), at concentrations at least 1000 times higher than used in the experiments and serially diluted in Krebs solution to the required final bath concentration.

Statistical analysis

Measured values were expressed as the mean ± s.e. of the mean. The n value refers to the number of preparations. Statistical significance was determined using either a paired or unpaired Student’s t test. Probabilities of less than 5% (P<0.05) were considered to be significant.

Results

Changes in body weight and blood glucose

All STZ-treated rats showed hyperglycemia at the time of the experiments, with their blood glucose concentration being significantly higher than those of the age-matched rats which were not injected with streptozotocin (normal rats) (normal 129 ± 6 mg/dL; STZ 485 ± 15 mg/dL, n=12, P<0.05). Therefore, STZ-treated rats were termed the STZ-induced diabetic rats. The
body weight of the STZ-induced diabetic rats were significantly lower than those of the age-matched normal rats (normal 409 ± 8 g; STZ 288 ± 17 g, n=12, P<0.05).

Mechanical responses produced by ET-1

In normal rats (n=12), circular smooth muscle strips of the gastric antrum maintained their resting tension at 0.16 ± 0.02 g and showed spontaneous oscillatory contractions with a frequency of 2.6 ± 0.1 min⁻¹ and an amplitude of 0.27 ± 0.02 g. In STZ-induced diabetic rats (n=12), the resting tension was 0.16 ± 0.01 g, and the frequency and the amplitude of the spontaneous contractions were 2.7 ± 0.1 min⁻¹ and 0.25 ± 0.02 g, respectively. These three parameters were not different significantly from those of the normal rats. The spontaneous oscillatory contractions were insensitive to TTX (1 µM; n=3), atropine (1 µM; n=3) and guanethidine (5 µM; n=3), indicating that they were myogenic in origin with no contribution of neuronal activity including cholinergic and adrenergic nerves.

ET-1 (10 nM) significantly elevated the resting tension (control: 0.17 ± 0.02 g; ET-1: 0.83 ± 0.08 g, n=11, P<0.05) and the frequency of spontaneous contractions (control: 2.6 ± 0.1 min⁻¹; ET-1: 2.9 ± 0.1 min⁻¹, n=11, P<0.05), but did not alter the amplitude of the spontaneous contractions (control: 0.28 ± 0.01 g; ET-1: 0.23 ± 0.02 g, n=11) (Fig. 1). In STZ-induced diabetic rats, ET-1 (10 nM) elevated the resting tension (control: 0.19 ± 0.02 g; ET-1: 0.97 ± 0.10 g, n=6, P<0.05), and the frequency (control: 2.5 ± 0.09 min⁻¹; ET-1: 2.8 ± 0.08 min⁻¹, n=6, P<0.05) of the spontaneous contractions was increased while the amplitude of the spontaneous contractions was decreased (control: 0.25 ± 0.02 g; ET-1: 0.13 ± 0.02 g, n=6, P<0.05) (Fig. 1).
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Effects of ET-1 on ET_A receptors

Experiments were carried out to investigate the effects of selective ET_A receptor activation, by applying ET-1 in the presence of BQ788 (1 \( \mu \text{M} \)), a selective antagonist of ET_B receptors. In normal rats, ET-1 significantly increased the tension (control: 0.19 ± 0.03 g; BQ788 1.01 ± 0.14 g, \( n=5, P<0.05 \)), decreased the amplitude (control: 0.56 ± 0.05 g; BQ788: 0.38 ± 0.04 g, \( n=5, P<0.05 \)), however did not alter the frequency (control: 2.0 ± 0.3 min\(^{-1}\); ET-1: 2.2 ± 0.2 min\(^{-1}\), \( n=5 \)) in the presence of BQ788 (Fig. 2). In diabetic rats, ET-1 increased both the resting tension (control: 0.13 ± 0.02 g; BQ788 0.74 ± 0.07 g, \( n=5, P<0.05 \)) and the amplitude (control: 0.33 ± 0.03 g; BQ788 0.82 ± 0.08 g, \( n=5, P<0.05 \)) of spontaneous contractions in the presence of BQ788. The frequency of spontaneous contraction was not altered in diabetic rats (control: 1.8 ± 0.1 min\(^{-1}\), \( n=5 \); ET-1: 2.1 ± 0.1 min\(^{-1}\), \( n=5 \)) (Fig. 2).

Effects of sarafotoxin S6c on ET_B receptors

The actions of ET_B receptor activation on gastric activity were examined by applying sarafotoxin S6c (S6c), a selective agonist of ET_B receptors. Interestingly, S6c showed quite different responses between normal rats and STZ-induced diabetic rats. In normal rats, S6c (10 nM) elevated the resting tension mildly (control: 0.17 ± 0.03 g; ET-1: 0.52 ± 0.04 g, \( n=8, P<0.05 \)). S6c also increased both the frequency (control: 2.7 ± 0.1 min\(^{-1}\); ET-1: 3.2 ± 0.1 min\(^{-1}\), \( n=8, P<0.05 \)) and amplitude (control: 0.24 ± 0.02 g; ET-1: 0.31 ± 0.04 g, \( n=8, P<0.05 \)) of spontaneous contractions in the presence of BQ788. In diabetic rats, ET-1 increased both the resting tension (control: 0.13 ± 0.02 g; BQ788 0.74 ± 0.07 g, \( n=5, P<0.05 \)) and the amplitude (control: 0.33 ± 0.03 g; BQ788 0.82 ± 0.08 g, \( n=5, P<0.05 \)) of spontaneous contractions in the presence of BQ788. The frequency of spontaneous contraction was not altered in diabetic rats (control: 1.8 ± 0.1 min\(^{-1}\), \( n=5 \); ET-1: 2.1 ± 0.1 min\(^{-1}\), \( n=5 \)) (Fig. 2).

Fig. 2. Effects of selective stimulation of ETA receptors with ET-1 on spontaneous oscillatory contractions in smooth muscle of the rat gastric antrum, in the presence of BQ788. N: Representative trace of the effect of ET-1 (10 nM) in the presence of BQ788 (1 mM) in normal rats. The resting tension of smooth muscle was 0.08 g. STZ: Representative trace of the effects of ET-1 in the presence of BQ788 in STZ-induced diabetic rats. The resting tension was 0.16 g.
contractions (Fig. 3). In contrast, S6c (10 nM) elevated the resting tension extremely in the STZ-induced diabetic rats (control: 0.18 ± 0.03 g; S6c, 1.84 ± 0.20 g, n=5, P<0.05). In STZ-induced diabetic rats, S6c tended to increase the frequency (control: 2.6 ± 0.2 min⁻¹; ET-1: 3.1 ± 0.1 min⁻¹, n=5) of spontaneous contractions, however this was not significant (Fig. 3). It was not possible to compare the amplitude of the spontaneous contractions, because the extreme elevation of the resting tone almost abolished the oscillation. Finally, to focus on the elevation of the resting tension through ET₅ receptors, the effects of S6c in STZ-induced diabetic rats was significantly greater than that in normal rats (normal rats: 3.7 ± 0.7 times of the control, n=8; STZ-induced diabetic rats: 11.2 ± 1.6 times of the control, n=5, P<0.05).

**Discussion**

The effects of ET-1 on the spontaneous oscillatory contraction of the diabetic gastric antrum were investigated in STZ-induced diabetic rats. In the resting condition, there was no difference in the amplitude and the frequency of spontaneous contractions between normal rats and STZ-induced diabetic rats. However, the effects of ET-1 were compared for three of the parameters of the spontaneous contractions, namely the resting tension and their frequency and amplitude. ET-1 elevated the resting tension and increased the frequency from the resting condition, but did not alter the amplitude in normal rats, while ET-1 elevated the resting tension and increased the frequency, but decreased the amplitude of spontaneous contractions in diabetic rats (Fig. 1). Attempts were made to examine a possible involvement of changes in the properties of the subtypes of ET receptors on the actions of ET-1, using selective agonists or antagonists. No
significant alteration in the actions of ET-1 on the ET$_A$ receptors was observed in STZ-induced diabetic rats. However, the actions of the ET$_B$ receptor stimulation by ET-1 were quite different between these two groups of rat, as only the STZ-induced diabetic rats showed an increased resting tension in response to S6c, an agonist of ET$_B$ receptors. The enhanced elevation of the resting tension level almost abolished the oscillatory contractions, and as a consequence the changes in amplitude of the oscillations were difficult to estimate.

Although previous studies have reported that spontaneous activity of the smooth muscle in the gastrointestinal tract was attenuated in diabetic-model animals (Xue and Suzuki, 1997; Takano et al., 1998; Imaeda et al., 1998; Ordog et al., 2000), no such alteration was observed in the present study. The elevation of blood glucose levels and the loss of body weight observed in the present study were comparable to those previously reported by others. It remains unclear why no alteration in the rhythmicity of gastric spontaneous activity during diabetic development was observed in the present study. Some differences between this study and those of others need to be considered, such as the difference in species (mice versus rats), the mechanism of diabetes development (genetic versus drug), the parameters measured (electrical activity versus mechanical activity) or the method used for the application of STZ (intravenous versus intraperitoneal). It remains unclear whether these differences are indeed responsible for the alteration of spontaneous activity in the stomach of STZ-induced diabetic rats.

ET-1 modulates both the frequency of slow waves and the resting membrane potential, through different types of ET receptors; ET-1 accelerates the frequency of slow waves and depolarizes the membrane through ET$_B$ receptors, while, ET-1 hyperpolarizes the membrane through ET$_A$ receptors by the activation of small conductance Ca$^{2+}$-sensitive K$^+$ channels (Imaeda et al., 2004). Interestingly, Endo et al. (2005) have reported that the contractile response to ET-1 is increased in the gastric fundus due to the increased density of ET$_B$ receptors in STZ-induced diabetic rats. This also seems to be the case in the present study. In STZ-induced diabetic rats, S6c produced much greater elevation of the resting tension than it did in normal rats, indicating that the action of ET$_B$-receptors was increased in the diabetic condition. Possible mechanisms for the increased response to ET-1 such as that the ET$_B$ receptors have become hypersensitive or that there has been an increase in the density of ET$_B$ receptors must be considered. However, it remains unclear why the increase in frequency of spontaneous contraction by ET-1 was not significant in STZ-induced diabetic rats, unlike the changes in the responses of resting tension. Our previous study demonstrated that ET-1 depolarized the membrane through ET$_B$ receptors by the activation of Ca$^{2+}$-activated Cl$^-$ channels and accelerated the frequency of slow waves independently of depolarization (Imaeda et al., 2004). In the present study, the former action, that is depolarization by activation of Ca$^{2+}$-activated Cl$^-$ channels, may be facilitated, while the latter action, that is the acceleration of the frequency, may not be altered by development of diabetes mellitus. It has been demonstrated that the mRNA expression level for ET$_B$ receptors was significantly increased in the diabetic stomach fundus (Endo et al., 2005). Thus, one possibility is that an increased level of expression of ET$_B$ receptors in the smooth muscle cells of the antrum facilitated the activation of Ca$^{2+}$-activated Cl$^-$ channels, resulting in the depolarization and increase of tension. However, an increased level of ET$_B$ receptors in smooth muscle cells may not be responsible for the accelerated frequency. It
is generally considered that two types of ICC, namely ICC in the myenteric plexus (ICC-MY) and intramuscular ICC (ICC-IM) (Komuro et al., 1996; Burns et al., 1997), are responsible for the determination of rhythmicity in gastro-intestinal smooth muscle. Studies using W/Wv mutant mice have revealed that ICC-MY are predominant in the generation of slow waves while ICC-IM are responsible for the generation of the regenerative component of slow waves (Ward et al., 1994; Huizinga et al., 1995; Dickens et al., 1999; 2001; Kito and Suzuki, 2003). Taken together, it could be considered that the action of ET-1 on ICC-MY and/or ICC-IM through ETB receptors may result in the acceleration of the frequency of spontaneous activity in the gastric antrum.

In summary, ET-1 elevates the resting tension and increases the frequency of spontaneous oscillatory contraction in both normal and STZ-induced diabetic rats similarly. The elevation of the tension via ETB receptor is significantly greater in the STZ-diabetic rats than that in the normal rats. The facilitation of ETB receptor signaling in the antrum may be involved in the pathogenesis of diabetic gastropathy.

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References


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