British Journal of Pharmacology (1995) 116, 1551-1556

Effect of diabetes on relaxations to non-adrenergic, non-cholinergic nerve stimulation in longitudinal muscle of the rat gastric fundus

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1 The effect of 8-week streptozotocin-induced diabetes has been examined on relaxations to non-adrenergic, non-cholinergic (NANC) nerve stimulation in longitudinal strips of rat gastric fundus.

2 In the presence of noradrenergic and cholinergic blockade and raised tissue tone, electrical field stimulation (0.5-4 Hz, 30 s trains) induced frequency-dependent relaxations that were significantly smaller in gastric fundus strips from diabetic rats than in strips from control rats.

3 N^G-nitro-L-arginine methyl ester (NAME, 100 μM) significantly reduced NANC relaxations in muscle strips from both control and diabetic rats, but the reduction was greater in muscle strips from diabetic rats than in those from control rats at frequencies of 2 and 4 Hz. α-Chymotrypsin (1 u ml^{-1}) slightly reduced relaxations to nerve stimulation in muscle strips from both control and diabetic rats.

4 The duration of NANC nerve relaxations (1-4 Hz, 30 s trains) was smaller in muscle strips from diabetic rats than in those from control rats. The duration of NANC relaxations was reduced by α-chymotrypsin (1 u ml^{-1}) in muscle strips from control rats but not in muscle strips from diabetic rats.

5 Relaxations to both nitric oxide (NO; 1-30 μM) and vasoactive intestinal polypeptide (VIP; 0.1-30 μM) were concentration-dependent and did not differ between muscle strips from control and diabetic rats.

6 The results suggest that streptozotocin-induced diabetes impairs relaxations to NANC nerve stimulation in the rat gastric fundus, which are largely mediated by NO and to a lesser extent by VIP. The impairment appears to occur at the prejunctional level, as smooth muscle reactivity to NO and VIP is not altered.

Keywords: Autonomic neuropathy; diabetes; nitric oxide; non-adrenergic non-cholinergic nerves; rat gastric fundus; streptozotocin; vasoactive intestinal polypeptide

Introduction

Patients with diabetes mellitus can suffer a range of gastrointestinal symptoms including chronic diarrhoea and *gastroparesis diabeticorum*, a term used to describe gastric atony associated with delayed gastric emptying and increased gastric residual volume (Kassander, 1958; Falchuk & Conlin, 1993). There is a strong correlation between the development of diabetic autonomic neuropathy, a characteristic complication of diabetes, and that of diabetic diarrhoea and diabetic gastroparesis (Falchuk & Conlin, 1993; Kawagishi *et al.*, 1994). Further, diabetic patients with gastroparesis have the same clinical and radiographic presentation as patients who have undergone vagotomy, so it is likely that visceral autonomic neuropathy is important in the aetiology of diabetic gastrointestinal complications (Scarpello & Sladen, 1978; Falchuk & Conlin, 1993).

There is abundant evidence for autonomic neuropathy in the streptozotocin-induced diabetic rat model. Functional, neurochemical and histochemical studies have demonstrated a complex range of cholinergic, adrenergic and peptidergic neurotransmitter alterations in the gastrointestinal tract of diabetic rats. Histological abnormalities have been observed for nerve fibres and varicosities in the intestinal myenteric plexus (Lincoln *et al.*, 1984; Belai *et al.*, 1985; Belai & Burnstock, 1990). Impaired neuromuscular transmission (Nowak *et al.*, 1986; D'Amato & Currò, 1990), impaired smooth muscle responsiveness to neurotransmitters (Lucas & Sardar, 1991), and abnormal gastrointestional content and release of neurotransmitters (Ballmann & Conlon, 1985; Belai *et al.*, 1985; 1987; 1988; 1991a,b) have all been reported. Furthermore, the neuronal effects of diabetes can vary with the neurotransmitter involved (Belai *et al.*, 1985; 1987; 1988), or between different regions or nerve plexus of the gastrointestinal tract (Belai *et al.*, 1985; 1991b; Belai & Burnstock, 1990) in the streptozotocin-induced diabetic rat.

Nitric oxide (NO) has a primary role as an inhibitory non-adrenergic, non-cholinergic (NANC) neurotransmitter at sites throughout the gastrointestinal tract (Rand, 1992; Sanders & Ward, 1992). Intravenous injection of a specific inhibitor of NO synthesis has been shown to delay gastric emptying of a non-nutrient solution in rats, demonstrating a physiological role of NO in the regulation of gastric emptying (Plourde *et al.*, 1994). The effect of diabetes on NO-mediated (or nitrergic) neurotransmision in the gastrointestinal tract has not previously been reported, however, our laboratory has demonstrated impaired nitrergic neurotransmission in the anococcygeus muscle from 8-week diabetic rats (Way & Reid, 1994a,b). The aim of the present study was to examine the effect of diabetes on nitrergic neurotransmission in a gastrointestinal tissue, the rat gastric fundus, in which the inhibitory NANC neurotransmission is mediated by NO and vasoactive intestinal polypeptide (VIP; Li & Rand, 1990; Boeckxstaens *et al.*, 1991; D'Amato *et al.*, 1992).

Methods

Diabetic model

Male Sprague-Dawley rats (180-200 g) were lightly anaesthetized with sodium pentobarbitone (40 mg kg^{-1}, i.p.). Diabetes was then induced by a single injection via the tail vein of streptozotocin (STZ; 65 mg kg^{-1}) dissolved in 20 mM citrate buffer vehicle (pH 4.5). The STZ-treated rats received 2% sucrose in their drinking water for the first 48 h after treatment

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to reduce the severity of the initial hypoglycaemic phase following STZ injection. Thereafter STZ-treated rats were maintained on rat chow and normal water *ad libitum*. Weight-matched vehicle-treated (control) rats were injected with citrate buffer only and fed rat chow and water throughout the study. The presence of glycosuria in STZ-treated rats was confirmed 1 week after treatment with Tes-tape urine sugar analysis paper (Lilly, U.S.A.). Rats were stunned and killed by decapitation 8 weeks after treatment, at which time a blood sample was collected for blood glucose analysis with an Ames glucometer II (Miles Laboratories, Australia).

Tissue preparation

The stomach was removed immediately after rats were killed, and two longitudinal strips were prepared from the ventral part of the fundus as described by Li & Rand (1990). Each fundus strip was mounted in a 6 ml water-jacketed organ bath under a resting tension of 1 g in physiological salt solution (PSS) of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.03, $MgSO_4$ 0.45, $NaHCO_3$ 25.0, D-(+)-glucose 11.1, disodium edetate 0.067 and ascorbic acid 0.14. The PSS was maintained at 37°C, gassed with 5% CO_2 in O_2, and contained atropine (3 μM) and guanethidine (5 μM) throughout the experiment to block cholinergic and noradrenergic responses to electrical field stimulation. Intramural nerves were electrically stimulated with two platinum wire electrodes, one placed on either side of the strip, with square wave pulses of 1 ms duration and supramaximal voltage (17 V cm^{-1}). Changes in tissue length were measured with an Ugo Basile isotonic transducer and recorded on a MacLab data acquisition system.

Experimental protocol

Each fundus strip was allowed to equilibrate for at least 30 min before 5-hydroxytryptamine (5-HT, 10 μM) was added to produce a sustained increase in tone. After a further 30 min equilibration period, a control concentration-response curve was obtained to either electrical field stimulation (EFS; 0.5–4 Hz, 30 s train), NO (1–30 μM) or VIP (0.1–30 nM). Relaxant responses to EFS (see Figure 1a) and NO (Figure 5a) were obtained at 5 min intervals in a non-cumulative manner, whereas relaxations to VIP (Figure 6a) were obtained in a cumulative manner.

A second concentration-response curve was then obtained in the absence (time-control experiments) or presence of N^G-nitro-L-arginine methyl ester (NAME, 100 μM) or α-chymotrypsin (1 u ml^{-1}). In some experiments a third concentration-response curve was also obtained in the presence of both NAME (100 μM) and α-chymotrypsin (1 u ml^{-1}) after the second curve in the presence of α-chymotrypsin. Responses obtained from the second and third curves have been expressed as a percentage of control responses obtained from the first curve in the same tissue. Tissues were exposed to NAME and/or α-chymotrypsin for approximately 20 min before responses were elicited. The length of fundus strips was measured under 1 g tension before the addition of 5-HT and the wet weight after blotting was determined at the end of the experiment.

Analysis of results

Data are expressed as means ± s.e.mean and n indicates the number of animals tested. Differences between means were assessed by Student's unpaired two-tailed t test, or by one-way multiple analysis of variance (MANOVA) followed by Student's t test. Linear regression analysis was performed by the method of least squares to investigate the relationship between magnitude and duration of responses. Probability values less than 0.05 ($P<0.05$) were taken to indicate statistical significance.

Drugs and drug solutions

The following drugs were used in the study: atropine sulphate (Sigma, U.S.A.), α-chymotrypsin (Sigma, U.S.A.), guanethidine sulphate (Ciba-Geigy, Australia), 5-hydroxytryptamine creatinine sulphate (serotonin; Sigma, U.S.A.), nitric oxide gas (NO; CIG, Australia), N^G-nitro-L-arginine methyl ester (NAME; Sigma, U.S.A.), pentobarbitone sodium (Boehringer Ingelheim, Australia), streptozotocin and tetrodotoxin (Sigma, U.S.A.), vasoactive intestinal polypeptide (VIP, human; Auspep, Australia). Saturated solutions of NO (2 mM) were prepared on the day of the experiment as described by Feelisch (1991). Briefly, vials of deionized water, deoxygenated by bubbling with argon gas for 1 h, were bubbled with NO gas for 20 min to give saturated solutions of NO.

Results

At the time of death, blood glucose levels in STZ-treated rats (26.1 ± 1.1 mM, $n=22$) were significantly elevated ($P<0.05$, unpaired t test) when compared to those in vehicle-treated rats (6.8 ± 0.2 mM, $n=25$). Weight gain over the 8-week post-treatment period was significantly less ($P<0.05$, unpaired t test) in STZ-treated rats (101.1 ± 12.1 g, $n=22$) than in vehicle-treated rats (342.1 ± 7.2 g, $n=25$). The stomach and intestines of STZ-treated rats were greatly distended at the time of death.

The length (20.7 ± 0.4 mm, $n=22$) and weight (0.9 ± 0.0 g, $n=22$) of gastric fundus strips dissected from STZ-treated rats were not different ($P>0.05$, unpaired t test) from those of fundus strips from vehicle-treated rats (19.8 ± 0.5 mm and 0.9 ± 0.0 g, $n=25$). Contractions to 5-HT (10 μM) did not differ in gastric fundus strips from STZ-treated (10.7 ± 0.5 mm, $n=22$) and vehicle-treated (10.9 ± 0.5 mm, $n=25$) rats ($P>0.05$, unpaired t test), and were well maintained throughout experiments. Therefore relaxant responses were examined under similar conditions for tissues from both groups of rats.

Magnitude of relaxations to NANC nerve stimulation

EFS (0.5–4 Hz, 30 s train) produced frequency-dependent relaxations in fundus strips from both STZ- and vehicle-treated rats (Figure 1); the relaxations were abolished by a 10 min exposure to tetrodotoxin (1 μM, data not shown), confirming that they were neuronal in origin. The magnitude of relaxations to EFS was greatly reduced in fundus strips from STZ-treated rats when compared to those from vehicle-treated rats at all frequencies tested (Figure 1).

The NO-synthase inhibitor NAME (100 μM) markedly reduced the magnitude of responses to EFS in fundus strips from STZ- and vehicle-treated rats (Figure 2a); the concentration of NAME used (100 μM) was maximally effective, since 300 μM NAME did not produce a greater inhibition of EFS-induced responses (data not shown). NAME inhibited responses to EFS at 2 and 4 Hz to a greater extent in fundus strips from STZ-treated rats than in strips from vehicle-treated rats (Figure 2a). The peptidase α-chymotrypsin (1 u ml^{-1}) slightly but significantly reduced the magnitude of relaxations to EFS in tissues from STZ-treated rats at 0.5, 1 and 4 Hz, and vehicle-treated rats at 0.5 and 1 Hz (Figure 2b).

When used in combination, NAME and α-chymotrypsin greatly reduced the magnitude of responses of EFS in fundus strips from both STZ- and vehicle-treated rats (data not shown; $P<0.05$, one-way MANOVA); the effect was not different from that of NAME alone in either group of rats ($P>0.05$, one-way MANOVA).

Duration of relaxations to NANC nerve stimulation

To assess the duration of relaxations to EFS, the mean time taken for an 80% reduction in the magnitude of the peak response was measured (T_{80}, see Figure 3). The duration of re-

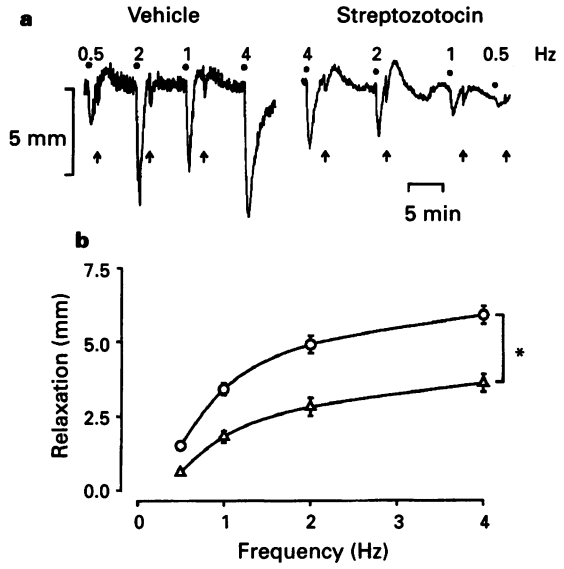

Figure 1 (a) Original traces showing relaxant responses to electrical field stimulation (●; 0.5–4 Hz, 30 s trains) in longitudinal strips of gastric fundus from vehicle- and streptozotocin-treated rats (↑ indicates wash). (b) Frequency-response curves for relaxant responses to electrical field stimulation (0.5–4 Hz, 30 s trains) in fundus strips from vehicle (○)- and streptozotocin (△)-treated rats. Values are means ± s.e.mean for 22–25 experiments. $^*P<0.05$ (one-way MANOVA).

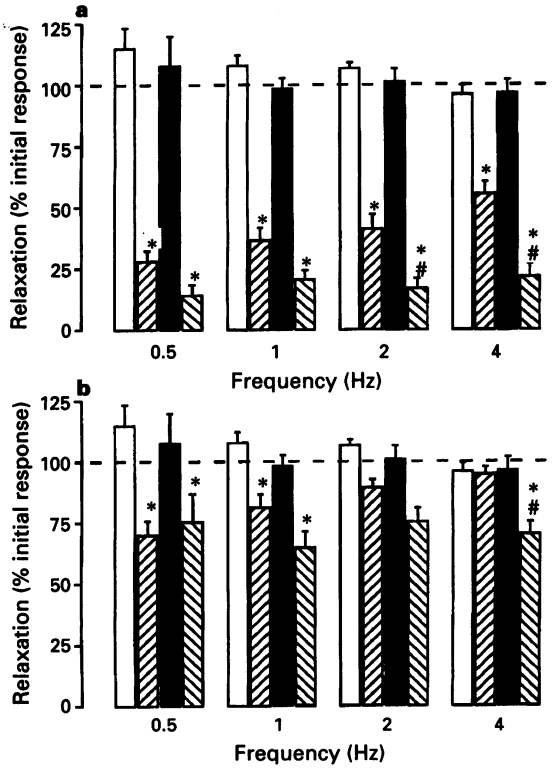

Figure 2 Effects of (a) N^G-nitro-L-arginine methyl ester (NAME, 100 μM) and (b) α-chymotrypsin (1 u ml^{-1}) on relaxant responses to electrical field stimulation (0.5–4 Hz, 30 s trains) in longitudinal strips of gastric fundus from vehicle (▨)- and streptozotocin (▧)-treated rats. Values are means ± s.e.mean for 10–14 experiments, expressed as percentages of initial responses obtained before the addition of (a) NAME or (b) α-chymotrypsin. Time controls (□ vehicle; ■ streptozotocin) indicate responses obtained after 90 min in the absence of NAME or α-chymotrypsin. $^*P<0.05$ versus corresponding time control (one-way MANOVA followed by Student's *t* test); $^\#P<0.05$ versus (a) vehicle NAME or (b) vehicle α-chymotrypsin (one-way MANOVA followed by Student's *t* test).

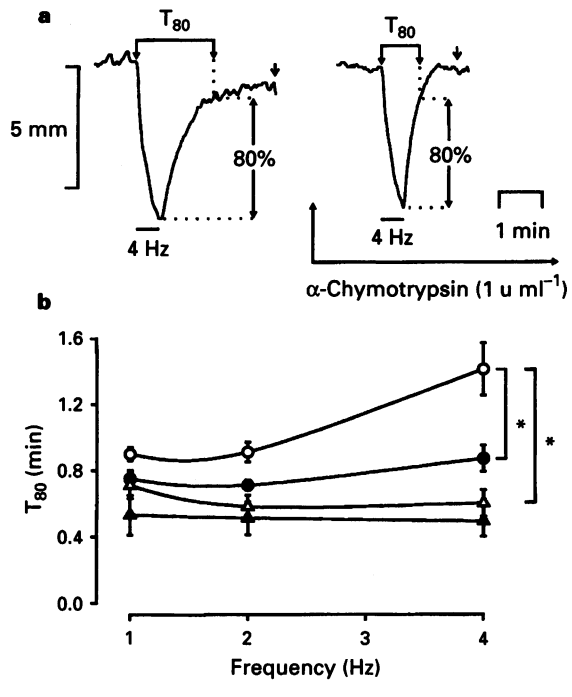

Figure 3 (a) Original trace showing the effect of α-chymotrypsin (1 u ml^{-1}) on the duration of relaxant responses to electrical field stimulation (4 Hz, 30 s train) in a longitudinal strip of gastric fundus from a vehicle-treated rat (↓ indicates wash). The duration of relaxant responses was measured as the mean time for an 80% reduction in the magnitude of the peak response (T_{80}). (b) Frequency-response curves for the duration (T_{80}) of relaxant responses to electrical field stimulation (1–4 Hz, 30 s trains) in rat gastric fundus strips before (○ vehicle; △ streptozotocin) and after (● vehicle, ▲ streptozotocin) the addition of α-chymotrypsin (1 u ml^{-1}). Values are means ± s.e.mean for 8–11 experiments. $^*P<0.05$ (one-way MANOVA).

laxations to EFS (1, 2 and 4 Hz; 30 s train) was smaller in fundus strips from STZ-treated rats than in those from vehicle-treated rats (Figure 3). The reduced duration was not merely a consequence of the reduced magnitude of relaxations in strips from STZ-treated rats. Figure 4 illustrates that, for responses of similar magnitude, the duration of relaxations to EFS was generally shorter in strips from STZ-treated rats than in those from vehicle-treated rats. For example, when responses of between 3 and 5 mm in magnitude are selected (vehicle: 4.4 ± 0.2 mm, $n=6$; STZ: 3.9 ± 0.2 mm, $n=10$; $P>0.05$, unpaired *t* test), the duration of relaxations in STZ-treated rats (0.8 ± 0.1 min) is significantly less ($P<0.05$, unpaired *t* test) than that in vehicle-treated rats (1.8 ± 0.3 min).

α-Chymotrypsin (1 u ml^{-1}) reduced the duration of relaxations to EFS (1, 2 and 4 Hz) in tissues from vehicle-treated rats but had no significant effect on the duration of relaxations to EFS in STZ-treated rats (Figure 3). Since NAME (100 μM) almost abolished relaxations to EFS in fundus strips from STZ-treated rats (Figure 2a), its effect on the duration of responses was not examined.

Relaxations to exogenous NO

Addition of exogenous NO (1–30 μM) to pre-contracted fundus strips produced rapidly-developing concentration-dependent relaxations that did not differ between fundus strips from STZ- and vehicle-treated rats (Figure 5; $P>0.05$, one-way MANOVA). NAME (100 μM) did not affect responses to NO in tissues from either group of rats (data not shown, $n=4$; $P>0.05$, one-way MANOVA). α-Chymotrypsin (1 u ml^{-1}) did not affect relaxations to NO in tissues from vehicle-treated rats (Figure 5b; $P>0.05$, one-way MANOVA), but slightly and significantly reduced the magnitude of relaxations to NO (10 and 30 μM) in tissues from STZ-treated rats (Figure 5b).

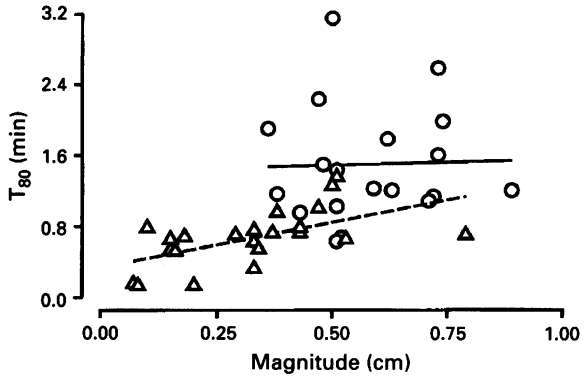

Figure 4 Relationship between the duration (T_{80}) and the magnitude of relaxant responses to electrical field stimulation (4 Hz) in gastric fundus strips from vehicle-treated (○) and STZ-treated (△) rats. Each point represents a response from an individual experiment. Linear regression of the data for each group gave a correlation coefficient of 0.10 with a slope of 0.14 for the vehicle-treated group (solid line; $P<0.05$; $n=19$), and a correlation coefficient of 0.57 with a slope of 1.0 for the STZ-treated group (dashed line; $P<0.05$; $n=22$).

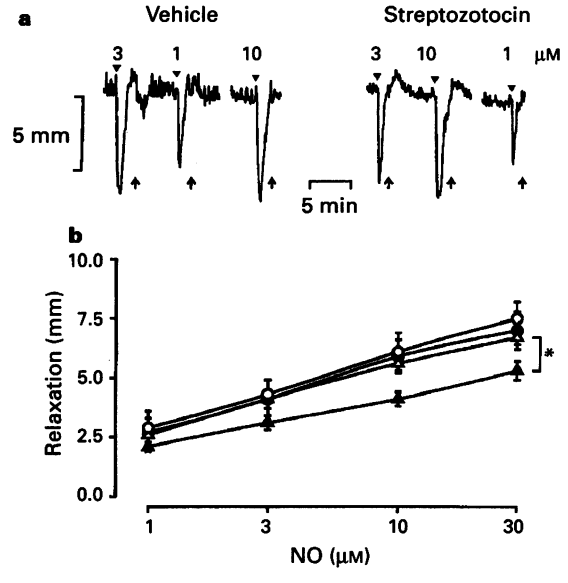

Figure 5 (a) Original traces showing relaxant responses to nitric oxide (NO, ▼; 1–10 μM) in longitudinal strips of gastric fundus from vehicle- and streptozotocin-treated rats (↑ indicates wash). (b) Frequency-response curves for relaxant responses to NO (1–30 μM) in gastric fundus strips before (○ vehicle; △ streptozotocin) and after (● vehicle; ▲ streptozotocin) the addition of α-chymotrypsin (1 u ml^{-1}). Values are means ± s.e.mean for 5 experiments. *$P<0.05$ (one-way MANOVA).

Relaxations to exogenous VIP

Exogenous VIP (0.1–30 nM) produced slowly-developing, well-maintained, concentration-dependent relaxations that did not differ between fundus strips from vehicle- and STZ-treated rats (Figure 6; $P>0.05$, one-way MANOVA). Relaxant responses to VIP were not affected by incubation with 100 μM NAME, but were abolished by 1 u ml^{-1}α-chymotrypsin (data not shown, $n=3$).

Discussion

This study has demonstrated that NANC relaxations to EFS are reduced in longitudinal strips of gastric fundus from 8-week streptozotocin-induced diabetic rats. Relaxant responses to the NANC neurotransmitters, NO and VIP, were not reduced in diabetic rat gastric fundus, suggesting that the impaired relaxations to NANC nerve stimulation are due to impaired release of neurotransmitters. These findings agree with those of D'Amato & Currò (1990) who showed that EFS-induced NANC relaxations (0.25–4 Hz) were reduced in diabetic rat gastric fundus 12 and 25 weeks after streptozotocin treatment, whereas responses to VIP were unaffected. In contrast, Belai *et al.* (1991a) reported that NANC relaxations to EFS (16 Hz, 2 min train) were not altered in gastric fundus from 8-week diabetic rats. There are two possible reasons for this discrepancy. Firstly, we have found that the magnitude of NANC relaxations to EFS at 16 Hz for 2 min in the rat gastric fundus is supramaximal (Jenkinson & Reid, unpublished observations), so differences in neuromuscular transmission between diabetic and control rat gastric fundus are less likely to be evident. Secondly, the balance of transmitters examined in our study would differ from that examined by Belai *et al.* (1991a), since NO is preferentially released at low stimulation frequencies and short train durations, whereas VIP release is greatly increased at higher frequencies and longer durations of stimulation in the rat gastric fundus (Li & Rand, 1990; D'Amato *et al.*, 1992).

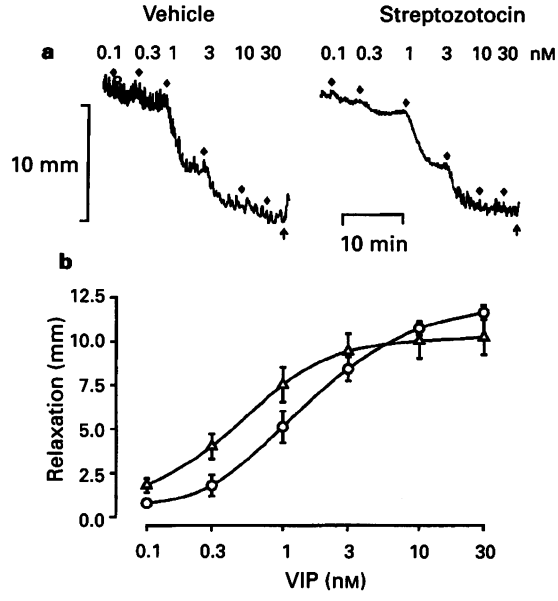

Figure 6 (a) Original traces showing relaxant responses to vasoactive intestinal polypeptide (VIP, ◆; 0.1–30 nM) in longitudinal strips of gastric fundus from vehicle- and streptozotocin-treated rats (↑ indicates wash). (b) Frequency-response curves for relaxant responses to VIP (0.1–30 nM) in fundus strips from vehicle (○)- and streptozotocin (△)-treated rats. Values are means ± s.e.mean for 5–7 experiments.

Way & Reid (1994a,b) demonstrated impaired nitrergic neurotransmission and a reduction in smooth muscle responsiveness to NO in the 8-week diabetic rat anococcygeus muscle, indicating impaired neuromuscular transmission at the post-junctional and possibly pre-junctional levels. In contrast, the present findings indicate only a prejunctional impairment to NANC neurotransmission in fundus strips from diabetic rats, as responses to NANC nerve stimulation were impaired whereas smooth muscle reactivity to NO and VIP was unaltered.

The inhibitory effects of NAME and α-chymotrypsin, when used alone and in combination at maximally effective concentrations, demonstrate the presence of three components of EFS-induced NANC relaxations in the rat gastric fundus: a NAME-sensitive or nitrergic component; an α-chymotrypsin-sensitive or VIP-like component; and a NAME- and α-chy-

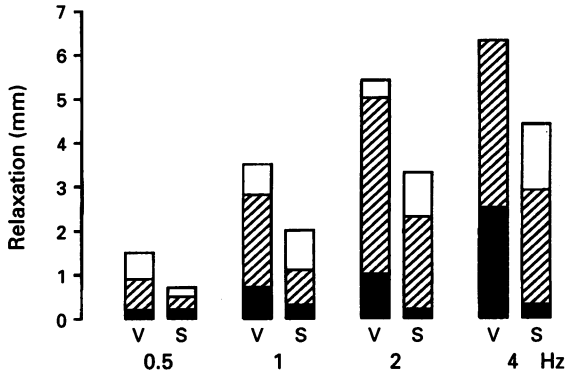

Figure 7 Magnitude of relaxant responses to electrical field stimulation (0.5–4 Hz, 30 s trains) in longitudinal strips of gastric fundus from vehicle (v)- and streptozotocin (s)-treated rats: division into α-chymotrypsin-sensitive component (□), N^G-nitro-L-arginine methyl ester (NAME)-sensitive component (▨), and NAME- and α-chymotrypsin-resistant component (■). The components of each response were determined by obtaining three consecutive sets of responses to field stimulation: (i) control responses (represented by full height of columns); (ii) responses in the presence of 1 u ml^{-1} α-chymotrypsin (□ = decrease in response); and (iii) response in the presence of 1 u ml^{-1} α-chymotrypsin and 100 μM NAME (▧ = further decrease in response). The relaxant response remaining in the presence of 1 u ml^{-1} α-chymotrypsin and 100 μM NAME is represented by ■. Values are means from 4–5 experiments.

motrypsin-resistant component, as previously suggested (Li & Rand, 1990; Belai *et al.*, 1991a; D'Amato *et al.*, 1992). Figure 7 illustrates the relative contribution of each of these components to the magnitude of NANC relaxations in tissues from control and diabetic rats. Belai *et al.* (1991) demonstrated that adenosine-5'-triphosphate is released upon EFS in the rat gastric fundus, and that its EFS-induced release is increased in the gastric fundus, of diabetic rats. However, in the present study the NAME- and α-chymotrypsin-resistant component of NANC relaxations is very small in diabetic rat gastric fundus compared to that in control tissues (Figure 7), suggesting that if a third transmitter is involved in these responses, its release is greatly reduced by diabetes. Work is currently being undertaken to examine the possible role of adenosine-5'-triphosphate as a NANC neurotransmitter in normal and diabetic rat gastric fundus.

NAME alone greatly reduced the magnitude of NANC relaxations in fundus strips from both control and diabetic rats. The effect of NAME was greater in fundus strips from diabetic rats than in strips from control rats (see Figure 2a), demonstrating that the nitrergic component of the NANC response is *proportionally* greater in the diabetic rat gastric fundus. In *absolute* terms, however, the nitrergic component of NANC responses in fundus strips from diabetic rats is smaller than that in strips from control rats (see Figure 7), suggesting that NO release is reduced in diabetic rat gastric fundus.

α-Chymotrypsin alone caused only a slight inhibition of the magnitude of NANC relaxant responses in fundus strips from both control and diabetic rats. However, the duration of NANC relaxations to EFS was much smaller in fundus strips from diabetic rats, and was greatly reduced by α-chymotrypsin in tissues from control but not diabetic rats. This demonstrates that VIP is important in mediation of the latter phase of NANC relaxations, as previously reported (Li & Rand, 1990; D'Amato *et al.*, 1992), and suggests that VIP release may be reduced in diabetic rat gastric fundus. Ballmann & Conlon (1985) reported that VIP content is decreased in the diabetic rat stomach. In contrast, Belai *et al.* (1991a) found no difference in stimulation (16 Hz, 2 min train)-induced release of VIP from control and diabetic rat gastric fundus; however, their findings are not comparable to those of our study, as previously discussed.

An unexpected finding in the present study was that α-chymotrypsin slightly reduced relaxations to NO in fundus strips from diabetic but not control rats, suggesting that NO may induce the release of VIP from diabetic rat gastric fundus strips. This correlates with previous reports that NO synthase inhibitors reduced the stimulation-induced release of VIP in guinea-pig gastric fundus (Grider *et al.*, 1992) and tonic release of VIP in canine ileum (Daniel *et al.*, 1994), and that the NO donor, sodium nitroprusside, stimulated VIP release in canine ileum (Daniel *et al.*, 1994). However, it is not known why this mechanism may be active in the gastric fundus from diabetic but not control rats. It is possible that the small reduction by α-chymotrypsin of the magnitude of NANC relaxations in diabetic rat gastric fundus may partly reflect inhibition of relaxations to NO-induced VIP release.

In gastric fundus strips from 2 of 22 diabetic rats, but from none of 25 control rats, the response to NANC nerve stimulation at 2 and 4 Hz was biphasic: an initial transient relaxation was followed by a large, rapidly developing contraction, demonstrating the EFS-induced release of a NANC contractile agent in these tissues. It is possible that the contractile agent mediating this response is released during NANC nerve stimulation in fundus strips from all diabetic rats but a contraction is not observed because of the opposing relaxant response. Work is currently being undertaken to investigate the possible involvement of contractile agents in NANC responses in gastric fundus strips from diabetic rats.

The physiological significance of a diabetes-induced impairment of NANC relaxations in the rat gastric fundus observed *in vitro* is unclear. *In vivo* evidence suggests a physiological role for NO in the modulation of gastric transit. Plourde *et al.* (1994) reported that intravenous injection of the NO-synthase inhibitor N^G-nitro-L-arginine caused a concentration-dependent delay in gastric emptying of a non-nutrient solution in rats. Furthermore, intravenous injection of N^G-nitro-L-arginine has been shown to decrease intragastric pressure in anaesthetized rats (Lefebvre *et al.*, 1992). In human subjects, gastric emptying of fluid is due mainly to fundic activity (Minami & McCallum, 1984). Therefore, the impairment in NANC relaxations demonstrated in longitudinal strips of rat gastric fundus may be important in disrupted gastric transit.

In summary this study has demonstrated reduced relaxations to NANC nerve stimulation but not to the neurotransmitters NO and VIP, in longitudinal strips of gastric fundus from 8-week streptozotocin-induced diabetic rats. The results indicate that the impaired relaxant response to NANC nerve stimulation is due to a reduction in the release of NO, VIP and possibly a third neurotransmitter, suggesting the presence of autonomic neuropathy in diabetic rat gastric fundus.

This work was supported by a Programme Grant from the National Health and Medical Research Council of Australia.

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(Received February 16, 1995
Revised May 12, 1995
Accepted May 17, 1995)