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Altered non-adrenergic non-cholinergic neurotransmission in gastric fundus from streptozotocin-diabetic rats

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Abstract

The influence of streptozotocin-induced diabetes has been investigated on responses to non-adrenergic, non-cholinergic (NANC) nerve stimulation in rat gastric fundus. NANC relaxations in precontracted muscle strips from diabetic rats were smaller than those from control rats. In addition, the relaxations in diabetic but not control rats were followed by rapidly-developing frequency-dependent contractions. In the presence of α-chymotrypsin and N^G-nitro-L-arginine methyl ester (L-NAME), the NANC contractions were markedly enhanced in diabetic rats. Treatment with the aldose reductase inhibitor, sorbinil, did not affect NANC relaxations or contractions in tissues from diabetic rats, and responses remained significantly different from those from control rats. The findings suggest that diabetes impairs relaxations to NANC nerve stimulation in the rat gastric fundus, and that a contractile NANC neurotransmitter(s) is released in diabetic rats. The results also suggest that diabetes-induced alterations in the NANC nerve response are not caused by increased activity of the aldose reductase pathway.

Keywords: Aldose reductase; Autonomic neuropathy; Diabetes; Non-adrenergic non-cholinergic; Gastric fundus, rat; Streptozotocin

1. Introduction

A common clinical complication in patients with diabetes mellitus is peripheral neuropathy affecting the motor, sensory, and autonomic nervous systems (Nathan, 1993). The streptozotocin-diabetic rat is a well-characterised experimental model of diabetes in which autonomic neuropathy has been widely studied (Schmidt et al., 1981; Sidenius, 1982). A complex range of functional, morphological and neurochemical alterations to adrenergic, peptidergic, purinergic, cholinergic, and serotonergic neurotransmission have been demonstrated in the gastrointestinal tract of streptozotocin-diabetic rats (Belai et al., 1987, 1988; D'Amato and Currò, 1990; Lincoln et al., 1984; Nowak et al., 1986). We have previously reported a functional impairment in the inhibitory non-adrenergic, non-cholinergic (NANC) neurotransmission in isolated strips of gastric fundus from diabetic rats (Jenkinson and Reid, 1995).

In the present study, we have further investigated the influence of 8-week streptozotocin-induced diabetes on the response to NANC nerve stimulation in the rat gastric fundus. In addition, we have examined two possible mechanisms that may underly diabetes-induced alterations of NANC neurotransmission in this tissue. Firstly, increased activity of the aldose reductase pathway may be involved. In certain tissues, including nerves, that are freely permeable to glucose, hyperglycaemia results in increased metabolism of glucose to sorbitol by the enzyme aldose reductase (Tomlinson et al., 1994). There is strong evidence that augmented activity of the aldose reductase pathway plays a role in the aetiology of diabetic neuropathy (Tomlinson et al., 1994). Increased intracellular accumulation of sorbitol has been demonstrated in peripheral nerves of streptozotocin-diabetic rats, coexistent with reduced motor nerve conduction velocity (Gillon et al., 1983; Mayer and Tomlinson, 1983; Tomlinson et al., 1982, 1984; Yue et al., 1982), neuroaxonal dystrophy (Schmidt et al., 1989), and abnormalities of axonal transport (Mayer and Tomlinson, 1983; Tomlinson et al., 1984). Furthermore, chronic treatment of diabetic rats with aldose reductase inhibitors normalises peripheral nerve sorbitol content

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concomitant with partial or full prevention of these neuronal abnormalities (Gillon et al., 1983; Mayer and Tomlinson, 1983; Schmidt et al., 1989; Tomlinson et al., 1982, 1984; Yue et al., 1982). One of the primary aims of the present study was to examine the possible involvement of increased polyol pathway activity in the altered NANC neurotransmission in the streptozotocin-diabetic rat gastric fundus, using the aldose reductase inhibitor sorbinil.

The second possibility that has been examined in the present study is that altered NANC neurotransmission in the diabetic rat gastric fundus is related to the acute change from hyperglycaemic conditions in vivo to relatively normoglycaemic conditions in vitro. To investigate this, we have examined NANC responses in isolated strips of gastric fundus from diabetic and control rats in in vitro medium where the in vivo glucose concentration of diabetic rats has been mimicked.

2. Materials and methods

2.1. Streptozotocin treatment

Diabetes was induced in male Sprague–Dawley rats (180–300 g) by a single injection via the tail vein of streptozotocin (65 mg/kg), dissolved in ice-cold 20 mM citrate buffer vehicle (pH 4.5) immediately before use. The streptozotocin-treated rats received 2% sucrose in their drinking water for the first 48 h after treatment to reduce the severity of the initial hypoglycaemic phase following streptozotocin injection, and thereafter received normal water ad libitum. Vehicle-treated (control) rats were injected with citrate buffer only and received normal water throughout the study. The presence of glycosuria in streptozotocin-treated rats was confirmed 1 week after treatment using Tes-tape urine sugar analysis paper (Lilly, Indianapolis, IN, USA). All rats were stunned and killed by decapitation 8 weeks after treatment, at which time a blood sample was collected for blood glucose analysis using an Ames glucometer 3 (Bayer Diagnostics, Mulgrave, Victoria, Australia).

2.2. Sorbinil treatment

A subgroup of streptozotocin-treated rats was maintained on sorbinil-supplemented rat chow throughout the study, commencing immediately after streptozotocin injection. All other rats were fed normal rat chow. Sorbinil-supplemented rat chow was prepared by the method used by Yue et al. (1982); briefly, sorbinil (0.5 mg/g), a small amount of distilled water and the binding agent gum xanthine (1% w/w), were added to crushed rat chow (GR2 +, Clark King, Preston, Victoria, Australia), and the mixture was dried in a laboratory oven at 37–42°C. The body weight and food consumption of sorbinil-fed rats were monitored daily, and the average daily sorbinil intake was 42 mg/kg body weight.

2.3. Tissue preparation

Immediately after rats were killed, the stomach was removed and the fundus dissected free and pinned flat. Two longitudinal strips (approximately 20 mm long by 3 mm wide) were prepared from the ventral part of the fundus by cutting parallel to the greater curvature as described by Li and 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 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. In some experiments, the physiological salt solution contained 30 mM D-(+)-glucose, as specified. The physiological salt solution was maintained at 37°C and gassed with 5% CO_2 in O_2. Intramural nerves were electrically stimulated using Grass S88 or S11 stimulators (Grass Instruments, Quincy, Mass, USA) via 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). The physiological salt solution contained atropine (3 μM) and guanethidine (5 μM) throughout the experiment to block cholinergic and noradrenergic responses to electrical field stimulation. Changes in tissue length were measured using a Ugo Basile isotonic transducer (Varese, Italy) and recorded using a MacLab data acquisition system (ADInstruments, Castle Hill, NSW, Australia). The length of fundus strips was measured under 1 g tension and resting tone, and the wet weight after blotting was determined at the end of experiments.

2.4. Experimental protocol

Each fundus strip was allowed to equilibrate for at least 30 min before serotonin (10 μM) was added to produce a sustained increase in tone. After a further 30-min equilibration period, control responses were obtained to electrical field stimulation (0.5–4 Hz, 30-s train) in random order at 5-min intervals.

A second set of responses was obtained in the presence of N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) and α-chymotrypsin (1 U/ml) together. Responses obtained from the second set of stimulations have been expressed as a percentage of control responses obtained from the first set in the same tissue. Tissues were exposed to L-NAME and α-chymotrypsin for at least 20 min before responses were elicited. In addition, control experiments were routinely carried out in the absence of L-NAME and α-chymotrypsin (time-control experiments), to demonstrate that responses did not vary over the time course of the experiment.

Table 1
Change in body weight, blood glucose levels, and fundus strip parameters for vehicle (20 mM citrate saline i.v.)-treated, streptozotocin (65 mg/kg i.v.)-treated, and streptozotocin and sorbinil (42 mg/kg/day p.o.)-treated rats
Values are means ± S.E.M. from the number of rats indicated.

	Vehicle	Streptozotocin	Streptozotocin and sorbinil
Number of rats	11	13	7
Change in body weight (g)[a]	+336.2±17.7	−3.2±13.5[b]	−24.1±17.9[b]
Blood glucose (mM)[c]	6.1±0.1	22.2±0.6[b]	24.2±0.7[b]
Length of fundus strips (mm)	20.2±0.3	19.5±0.6	20.9±0.7
Weight of fundus strips (g)	0.77±0.03	0.73±0.04	0.77±0.03
Contractile response to 10 μM serotonin (mm)	11.0±0.5	10.1±0.7	11.1±0.4

[a] Change in body weight over the 8-week treatment period.
[b] Significant difference from vehicle-treated, $P < 0.05$ (one-way ANOVA followed by Student–Newman–Keuls test).
[c] Blood glucose was determined from blood collected at the time of decapitation.

In a separate series of experiments, responses to electrical field stimulation (0.5–4 Hz; 30-s train) in fundus strips from control and diabetic rats were obtained in physiological salt solution containing 30 mM-glucose and compared to those obtained in tissues from the same rats in physiological salt solution containing 11.1 mM glucose.

2.5. Analysis of results

Data are expressed as means ± S.E.M. and *n* indicates the number of animals tested. Differences between means were assessed by one-way analysis of variance (ANOVA) or multiple analysis of variance (MANOVA), followed by Student–Newman–Keuls test where appropriate. Analyses were performed using the statistical software package Sigma Stat 1.0 (Jandel Scientific, San Rafael, CA, USA). Probability values less than 0.05 ($P < 0.05$) were taken to indicate statistical significance.

2.6. Drugs and drug solutions

Atropine sulphate, α-chymotrypsin, guanethidine sulphate, 5-hydroxytryptamine creatinine sulphate (serotonin), L-NAME, streptozotocin and tetrodotoxin were purchased from Sigma (St. Louis, MT, USA). Sorbinil (CP 45,634) was kindly donated by Pfizer (Groton, CT, USA). All drugs added to the organ bath were dissolved in distilled water to give stock solutions of 10 mM, and dilutions were made in physiological salt solution.

3. Results

Diabetic rats and sorbinil-treated diabetic rats lost a small amount of weight over the 8-week duration of the study, whereas control rats gained weight (Table 1). Change in body weight of diabetic rats and sorbinil-treated diabetic rats over the 8-week period was significantly reduced when compared to that of control rats ($P < 0.05$, one-way ANOVA; Table 1); the initial body weight of rats did not differ between groups ($P > 0.05$, one-way ANOVA; data not shown). At the time of death, the blood glucose concentration of diabetic rats and sorbinil-treated diabetic rats was significantly greater than that of control rats ($P < 0.05$, one-way ANOVA; Table 1). Blood glucose concentration and change in body weight of diabetic rats did not differ from those of sorbinil-treated diabetic rats ($P > 0.05$, one-way ANOVA). Neither the length and weight of fundus strips nor the contractile response to serotonin (10 μM), differed between control rats, diabetic rats and sorbinil-treated diabetic rats ($P > 0.05$, one-way ANOVA; Table 1).

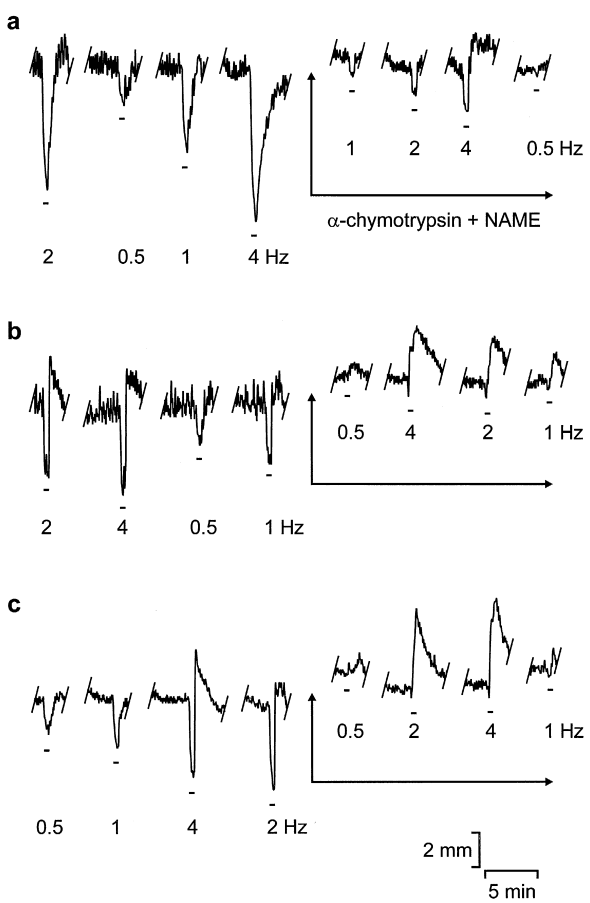

Fig. 1. Original traces showing the effect of L-NAME (100 μM) and α-chymotrypsin (1 U/ml) together on responses to non-adrenergic, non-cholinergic nerve stimulation (−; 0.5–4 Hz, 30-s trains) in longitudinal strips of gastric fundus from (a) control rats, (b) diabetic rats, and (c) sorbinil-treated diabetic rats.

3.1. Responses to NANC nerve stimulation

In fundus strips from all groups of rats, electrical field stimulation (0.5–4 Hz, 30-s train) produced frequency-dependent relaxations (Figs. 1 and 2) that remained consistent in time-control experiments. In tissues from 10 of 13 diabetic rats and from five of seven sorbinil-treated diabetic rats, but not in tissues from control rats, responses to electrical field stimulation (1–4 Hz) were biphasic: the initial relaxant response was followed by a frequency-dependent contraction that developed rapidly after electrical field stimulation was stopped (Figs. 1 and 3), and remained consistent in time-control experiments. Both relaxations and contractions to NANC nerve stimulation in tissues from all groups of rats were abolished by a 10-min exposure to tetrodotoxin (3 μM; data not shown). Tetrodotoxin did not affect the tone of tissues from any group of rats (data not shown).

The magnitude and duration (measured as the mean time taken for an 80% reduction in the magnitude of the peak response, T_{80}; see Jenkinson and Reid, 1995) of relaxant responses to electrical field stimulation in fundus strips from diabetic rats and sorbinil-treated diabetic rats, were greatly reduced ($P < 0.05$, one-way MANOVA) when compared with relaxations in tissues from control rats (Figs. 1, 2 and 4).

The magnitude of contractions to electrical field stimulation in fundus strips from diabetic rats and sorbinil-treated diabetic rats was significantly greater ($P < 0.05$, one-way MANOVA) than that in tissues from control rats (Figs. 1 and 3).

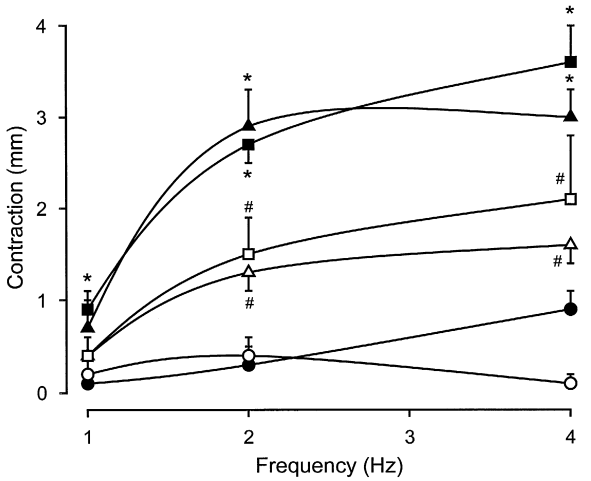

Fig. 3. Magnitude of contractile responses to electrical field stimulation (1–4 Hz, 30-s trains) in longitudinal strips of gastric fundus from control rats (circles), diabetic rats (triangles), and sorbinil-treated diabetic rats (squares) in the absence (open symbols) and presence (filled symbols) of L-NAME (100 μM) and α-chymotrypsin (1 U/ml). Values are means ± S.E.M. for four to five experiments. # Significant difference from control, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test). * Significant difference from control in the presence of L-NAME and α-chymotrypsin, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test).

The magnitude and duration of relaxations, and the magnitude of contractions to electrical field stimulation, did not differ ($P > 0.05$, one-way MANOVA) between tissues from diabetic rats and sorbinil-treated diabetic rats (Figs. 1–4

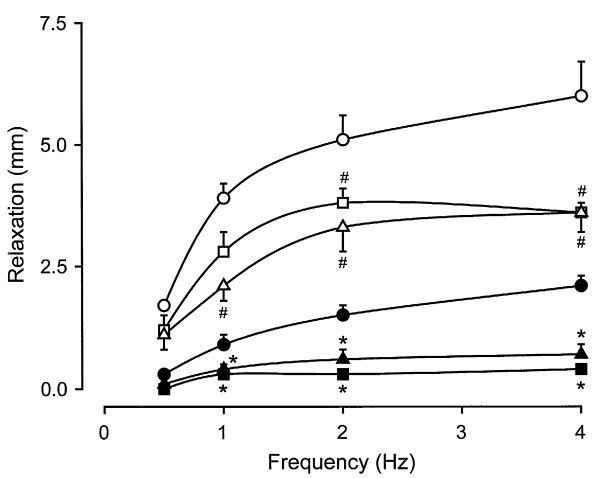

Fig. 2. Magnitude of relaxant responses to electrical field stimulation (0.5–4 Hz, 30-s trains) in longitudinal strips of gastric fundus from control rats (circles), diabetic rats (triangles), and sorbinil-treated diabetic rats (squares), in the absence (open symbols) and presence (filled symbols) of L-NAME (100 μM) and α-chymotrypsin (1 U/ml). Values are means ± S.E.M. for four to five experiments. # Significant difference from control, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test). * Significant difference from control in the presence of L–NAME and α-chymotrypsin, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test).

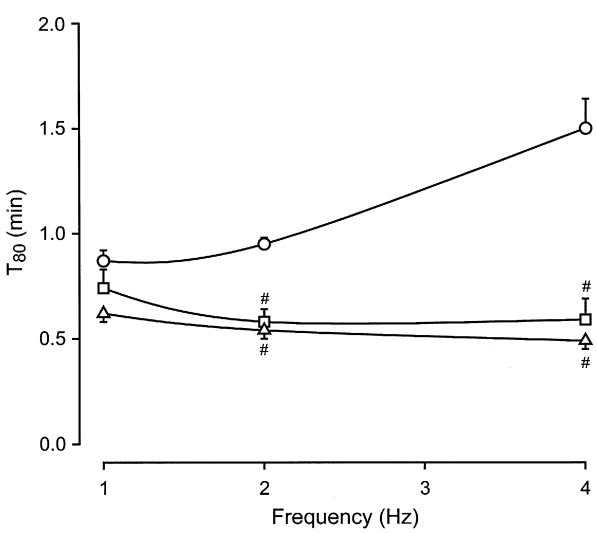

Fig. 4. Duration of relaxant responses to electrical field stimulation (1–4 Hz, 30-s trains) in longitudinal strips of gastric fundus from control rats (circles), diabetic rats (triangles), and sorbinil-treated diabetic rats (squares). The duration of relaxations was measured as the time taken for an 80% reduction in the magnitude of the peak response (T_{80}). Values are means ± S.E.M. for 7–13 experiments. # Significant difference from control, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test).

3.2. Effect of L-NAME and α-chymotrypsin on responses to NANC nerve stimulation

In combination, the nitric oxide synthase inhibitor, L-NAME (100 μM), and the peptidase, α-chymotrypsin (1 U/ml), greatly reduced ($P < 0.05$, one-way MANOVA) the magnitude of relaxations to electrical field stimulation (0.5–4 Hz; 30-s train) in fundus strips from the three groups of rats (Figs. 1 and 2). L-NAME and α-chymotrypsin almost abolished relaxations to electrical field stimulation in fundus strips from diabetic rats and sorbinil-treated diabetic rats and so their effect on the duration of relaxations was not examined.

L-NAME (100 μM) and α-chymotrypsin (1 U/ml) enhanced the magnitude of contractions to electrical field stimulation (1–4 Hz) in tissues from diabetic rats and sorbinil-treated diabetic rats, however the effect was significant ($P < 0.05$, one-way MANOVA) in tissues from diabetic rats only (Figs. 1 and 3). When tissues were incubated with L-NAME and α-chymotrypsin the contractile phase of responses to electrical field stimulation in fundus strips from 10 of 12 diabetic rats, and from seven of seven sorbinil-treated diabetic rats, developed during rather than after electrical field stimulation (Fig. 1), and was abolished by a 10-min exposure to tetrodotoxin (data not shown). In the presence of L-NAME and α-chymotrypsin, there were still no significant contractions to electrical field stimulation (1–4 Hz; $P > 0.05$, one-way MANOVA) in tissues from control rats, however a small rebound contraction following electrical field stimulation at 4 Hz was evident (Figs. 1 and 3).

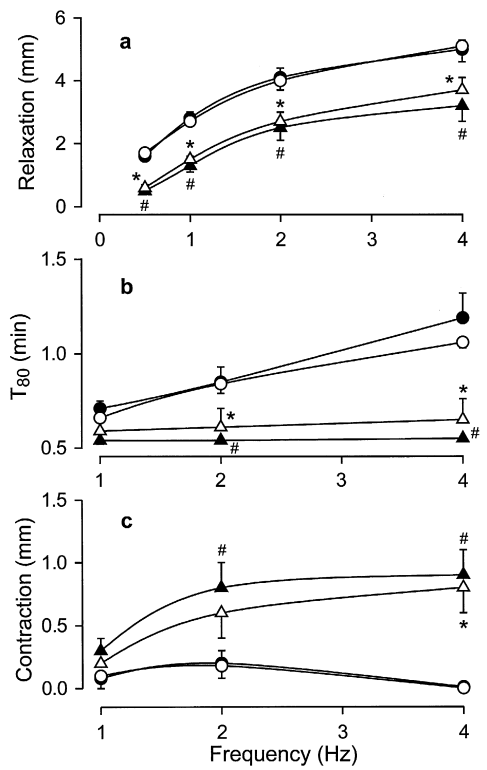

Fig. 5. (a) Magnitude and (b) duration of relaxant responses, and (c) magnitude of contractile responses to electrical field stimulation (0.5–4 Hz, 30-s trains) in longitudinal strips of gastric fundus from control (circles) and diabetic (triangles) rats, in medium containing 11.1 mM (open symbols)- or 30 mM (filled symbols)-glucose. The duration of relaxations was measured as the time taken for an 80% reduction in the magnitude of the peak response (T_{80}). Values are means ± S.E.M. for five experiments. * Significant difference from control in 11.1 mM glucose media, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test). # Significant difference from control in 30 mM glucose media, $P < 0.05$ (MANOVA, followed by Student–Newman–Keuls test).

3.3. Effect of 30 mM-glucose medium on responses to NANC nerve stimulation

In tissues from either control or diabetic rats, the magnitude and duration of relaxant responses to electrical field stimulation (0.5–4 Hz), and the magnitude of contractions to electrical field stimulation (1–4 Hz), obtained in 30 mM glucose medium (Fig. 5) did not differ ($P > 0.05$, one-way MANOVA) from those obtained in 11.1 mM glucose medium.

4. Discussion

The findings of the present study confirm our previous report that relaxant responses to NANC nerve stimulation are reduced in longitudinal strips of gastric fundus from 8-week streptozotocin-induced diabetic rats (Jenkinson and Reid, 1995). There are three components of electrical field stimulation-induced NANC relaxations in the rat gastric fundus: a nitric oxide-mediated component (L-NAME-sensitive); a vasoactive intestinal polypeptide-mediated component (α-chymotrypsin-sensitive); and a third, as yet unidentified, component (D'Amato et al., 1992; Jenkinson and Reid, 1995; Li and Rand, 1990). In our previous report, all three components of the NANC relaxation appeared to be reduced in diabetic rat gastric fundus: in absolute terms, the L-NAME-sensitive component of NANC relaxations was reduced in tissue from diabetic rats when compared to that from control rats, suggesting that the nitric oxide-mediated component of the response was reduced in diabetic rats; α-chymotrypsin had no effect on the duration of NANC relaxations in tissue from diabetic rats but greatly reduced their duration in tissue from control rats, suggesting that the vasoactive intestinal polypeptide-mediated component of NANC relaxations was reduced by diabetes; and, the residual relaxation in the presence of both L-NAME and α-chymotrypsin was greatly reduced in tissue from diabetic rats, as it was in the present study, suggesting that the third component of NANC relaxations was also reduced in fundus strips from diabetic rats. In contrast, relaxations to exogenous nitric oxide and vasoactive intestinal polypeptide, the two known inhibitory NANC neurotransmitters in this tissue, were unaltered (Jenkinson and Reid, 1995). The findings from both stud-

ies suggest that electrical field stimulation-induced release of each of the inhibitory NANC neurotransmitters is impaired in longitudinal strips of gastric fundus from streptozotocin-diabetic rats.

The present study has demonstrated that in fundus strips from diabetic but not control rats, the NANC response to electrical field stimulation is biphasic, the initial relaxation being followed by a large, rapidly developing contraction which appeared after electrical field stimulation had been stopped. Both the relaxant and contractile phases of the NANC response in the diabetic rat gastric fundus were abolished by tetrodotoxin, demonstrating that both are neuronal in origin. In the presence of L-NAME and α-chymotrypsin, the relaxant phase of responses to electrical field stimulation was almost abolished, whereas the contractile response was markedly enhanced and now developed rapidly during electrical field stimulation, in diabetic rat gastric fundus. The findings suggest that NANC relaxations in diabetic rat gastric fundus are actively opposed by the release of a contractile neurotransmitter(s) not usually evident in tissues from control rats, and that release of this contractile transmitter is greatly enhanced by diabetes. Therefore, it is likely that the impairment to NANC relaxations in diabetic rat gastric fundus is due to a combination of impaired release of inhibitory NANC neurotransmitters, as we have previously suggested (Jenkinson and Reid, 1995), and a masking effect of the enhanced contractile component of the responses. The contractile response to electrical field stimulation was only evident in tissues from two of 22 diabetic rats in our previous study (Jenkinson and Reid, 1995) and is described in detail here for the first time. The reason for this discrepancy is not known. Work is currently being undertaken to determine the mediator of electrical field stimulation-induced contractile responses in diabetic rat gastric fundus.

In the present study, the aldose reductase inhibitor sorbinil (Peterson et al., 1979) was used to examine the possible involvement of increased aldose reductase activity in the development of altered NANC neurotransmission in the diabetic rat gastric fundus. Chronic treatment of diabetic rats with sorbinil throughout the 8-week study period failed to prevent or reduce the observed alterations to electrical field stimulation-induced NANC responses; NANC relaxations or contractions in fundus strips from sorbinil-treated diabetic rats were not different to those from diabetic rats. Although the effectiveness of sorbinil treatment was not assessed in the present study, oral doses of sorbinil between 0.5–47 mg/kg/day p.o. have been extensively used to inhibit aldose reductase in diabetic studies, and have previously been demonstrated to prevent abnormal tissue sorbitol accumulation in various tissues from diabetic rats (Peterson et al., 1979; Schmidt et al., 1989; Tomlinson et al., 1984; Yue et al., 1982; Ashizawa et al., 1997; Mizuno et al., 1999).

In agreement with the lack of effect of aldose reductase inhibition in the present study, chronic sorbinil treatment did not prevent impaired NO-mediated neurotransmission (Way and Reid, 1996), abnormal nerve function (Gillon et al., 1983), impaired sciatic nerve doppler flux (Tomlinson et al., 1998) or altered glomerular structure and function (Mauer et al., 1989; Tilton et al., 1991) in streptozotocin-diabetic rats. In addition, a lack of effect of other aldose reductase inhibitors has been reported: tolrestat did not improve decreased nerve blood flow and impaired sciatic nerve motor conduction velocity (Calcutt et al., 1994) or impaired unmyelinated sciatic nerve fibre regeneration (Yasuda et al., 1999) in diabetic rats; and ponalrestat failed to prevent reduced numbers of calcitonin gene-related peptide immunoreactive nerve fibres in the ileum of diabetic rats (Belai et al., 1996). In contrast, sorbinil treatment has been demonstrated to partially normalize impaired sciatic nerve motor conduction velocity (Tomlinson et al., 1984; Yue et al., 1982; Ashizawa et al., 1997; Mizuno et al., 1999), defective sciatic nerve axonal choline acetyltransferase transport (Tomlinson et al., 1984), and neuronal dystrophy of the superior mesenteric sympathetic ganglia (Schmidt et al., 1989), in streptozotocin-diabetic rats.

Results from clinical trials using aldose reductase inhibitors are also inconsistent. Treatment of diabetic patients with sorbinil has been shown to cause a small improvement in motor and sensory nerve conduction velocities (Judzewitsch et al., 1983), and to result in active regeneration and repair of sural-nerve myelinated fibers (Sima et al., 1988). However, in a number of other studies sorbinil treatment had no effect on motor or sensory nerve conduction velocities, or on a range of abnormal autonomic or sensory nerve function tests in diabetic patients (Lewin et al., 1984; Martyn et al., 1987; Young et al., 1983). It is likely that the pathogenesis of diabetic neuropathies involves a number of different interacting mechanisms (Nathan, 1993). If so, this would explain why the effect of aldose reductase inhibitor treatment on isolated indicators of neuropathy, such as impaired nerve conduction velocity, and on symptomatic neuropathies in diabetic patients, varies greatly between studies.

It has been suggested that diabetes-induced impairments observed in vitro in normoglycaemic medium may be a manifestation of an acute change from in vivo hyperglycaemia to in vitro normoglycaemia. In support of this suggestion, Hodgson and King (1992) reported that reduced responsiveness to endothelin-1 in aortic rings from 2-week streptozotocin-diabetic rats was not observed when responses were obtained in medium containing 30 mM glucose. In the present study, NANC responses in gastric fundus strips from 8-week diabetic rats did not differ between 11.1 mM- and 30 mM-glucose medium, indicating that the altered responses to electrical field stimulation observed in vitro in diabetic rat gastric fundus are not the result of an acute change in glycaemic conditions. This correlates with previous reports that high glucose medium did not improve reduced responses to endothelin-1 in aortic rings from 6-week streptozotocin-diabetic rats

(Hodgson and King, 1992), or affect increased nerve hypoxic resistance in 2-month streptozotocin-diabetic rats (Cameron and Cotter, 1992).

In conclusion, the findings of the present study support our previous observation that streptozotocin-induced diabetes impairs the electrical field stimulation-induced release of inhibitory NANC neurotransmitters in the rat gastric fundus. Furthermore, the present results suggest that diabetes enhances the release of a contractile NANC neurotransmitter that is not normally apparent in tissues from control rats. The altered response to NANC nerve stimulation in tissues from diabetic rats was not prevented by chronic treatment with the aldose reductase inhibitor sorbinil, demonstrating that the alterations are not the result of increased activity of the aldose reductase pathway.

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