The Effectiveness of Treatments of Diabetic Autonomic Neuropathy Is Not the Same in Autonomic Nerves Supplying Different Organs

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The aim of the study was to investigate antioxidant (α-lipoic acid [LA]) and γ-linolenic acid treatments in the prevention of changes in autonomic nerves induced in streptozotocin-diabetic rats. Autonomic nerves supplying the heart, penis, and gut were examined using immunohistochemical and biochemical techniques. LA and γ-linolenic acid (present in evening primrose oil [EPO]) were administered as dietary supplements (−80 and 200 mg · kg−1 · day−1, respectively). LA treatment prevented the diabetes-induced decrease of norepinephrine (NA) in the heart and of type I nitric oxide synthase (NOS-I) expression in erectile tissue of the penis but failed to prevent diabetes-induced changes in NA-, vasoactive intestinal polypeptide–, or calcitonin gene–related peptide-containing nerves supplying the ileum. LA partially prevented and EPO totally prevented the increase in NOS-I activity induced by diabetes in the ileum. EPO treatment failed to prevent any other diabetes-induced changes in the heart, penis, or ileum. These results demonstrate that, whereas LA treatment is more effective than EPO in preventing diabetes-induced changes in autonomic nerves, the effectiveness of LA treatment varies with the target organ studied. Diabetes-induced changes in nerves supplying the ileum are more resistant to treatment than those of the heart and penis. Diabetes 52:157–164, 2003

It is recognized that autonomic neuropathy occurs as a complication of diabetes; indeed, diabetes is the most common cause of autonomic neuropathy (1). The autonomic innervation of the cardiovascular, gastrointestinal, and urogenital systems can all be affected, and autonomic neuropathy in diabetes causes a variety of symptoms, including postural hypotension, gastroparesis, disordered gut motility, bladder dysfunction, and impotence (2,3). Animal models, particularly the streptozotocin-diabetic rat, have been used extensively to investigate the autonomic nervous system in diabetes. In previous studies, we have shown that the pattern of change occurring in autonomic nerves in diabetes is complex. Within the same peripheral target, individual nerve populations, identified by their neurotransmitter content, respond differently. Some populations undergo a degenerative process, some have altered neurotransmitter content without evidence of degeneration, and others are relatively unaffected (4). Furthermore, the same nerve type may or may not undergo degeneration depending on the target it innervates (5).

A number of theories have been proposed to account for neuropathy in diabetes, including activation of the polyol pathway, depletion of myoinositol, impaired fatty acid metabolism, reduction in nerve blood supply, production of advanced glycation end products, oxidative stress, and inadequate trophic support (6–8). There is evidence for all these theories, and they are not mutually exclusive since several of the above factors can lead to oxidative stress by alterations in different metabolic pathways (9–11). As a consequence, both antioxidants (11–13) and the free fatty acid, γ-linolenic acid (14,15), have been investigated for the treatment of diabetic neuropathy, particularly with respect to nerve conduction and blood-flow deficits. However, their effectiveness in the treatment of autonomic neuropathy has received less attention.

Since we have demonstrated that diabetes can have a differential effect on subpopulations of autonomic nerves, it is important to establish whether therapeutic agents are equally effective in preventing autonomic neuropathy in different subpopulations. In this study, the effect of dietary supplementation with evening primrose oil (EPO), a source of γ-linolenic acid, and α-lipoic acid (LA), an antioxidant, in preventing diabetes-induced changes in autonomic nerves supplying three target organs was investigated using streptozotocin-diabetic rats. Autonomic nerves supplying the heart, penis, and ileum, representative of the cardiovascular, urogenital, and gastrointestinal systems, respectively, were investigated by immunohistochemical and biochemical techniques. Some of the data reported here have been presented in abstract form (16,17).

RESEARCH DESIGN AND METHODS

Animal groups and treatments. Adult male Wistar rats (weight 350–400 g) were divided into six groups: 1 and 2 Untreated diabetic (UD) and control (UC) rats consisted of rats receiving streptozotocin (65 mg/kg i.p.) or vehicle, respectively, maintained on a standard powdered diet; 3 and 4) LA-treated...
control (LAC) and diabetic (LAD) rats received LA (β,γ-thiolic acid; Sigma, Poole, U.K.) as a dietary supplement to give an approximate dose of 80 mg · kg⁻¹ · day⁻¹ (18). Food intake was monitored and the composition of the diet was adjusted to take into account the lower food intake of diabetic rats. EPO-treated control (EPOC) and diabetic (EPOD) rats received EPO (Efanol, Guildford, U.K.) as a dietary supplement to give an approximate dose of γ-linolenic acid of 200 mg · kg⁻¹ · day⁻¹ (19). All animals were maintained for 8 weeks and then killed by CO₂ asphyxiation. Blood samples were taken for glucose analysis (Glucotrend; Roche Diagnostics, Barcelona, Spain). The atria, penis, and ileum were dissected and processed for immunohistochemical and biochemical analysis. All procedures complied with U.K. legislation governing the use of animals in scientific research.

**Immunohistochemical analysis**

**Cryostat sections.** Samples of penile and ileum were mounted in optimum cutting temperature compound and frozen in liquid nitrogen—cooled isopentane. Cryostat sections (12 μm) were fixed for 30 min in 4% formaldehyde or 4% paraformaldehyde. After washing in PBS, sections were processed for the localization of type I nitric oxide synthase (NOS-I), vesicle acetylcholine transporter (VACHT), and tyrosine hydroxylase (TH) using the following polyclonal primary antibodies: rabbit anti-NOS-I (Santa Cruz Biotechnology, Santa Cruz, CA), goat anti-VACHT (Chemicon International, Temecula, CA), and rabbit anti-TH (Affiniti Research Products, Manhhead, U.K.) at dilutions of 1:500, 1:1,000, and 1:100, respectively. Sections were incubated with primary antibodies for 15 h at room temperature. For localization of NOS-I and TH, sections were washed in PBS and incubated with fluorescein isothiocyanate–conjugated goat anti-rabbit IgG (dilution 1:100; Nordic Immunological Laboratories, AA Tilburg, the Netherlands) for 90 min in the dark. After washing, sections were counterstained with 0.1% pontamine sky blue in 1% dimethylsulphoxide (wt/vol in PBS) for 5 min and mounted in citifluor. For the localization of VACHT, sections were processed as above except that Cy3-conjugated donkey anti-sheep IgG (1:100; Jackson ImmunoResearch, West Grove, PA) was used as the secondary antibody and pontamine sky blue counterstaining was omitted.

**Wholomeat preparations.** Segments of ileum were opened, cleaned, pinned on sylgard, and fixed in 4% paraformaldehyde or 4% formaldehyde for 2.5 h. After washing, samples (~0.5 cm²) were cut and the muscle layers with the myenteric plexus were peeled away. The smooth muscle/myenteric plexus preparations were placed, circular muscle side down, on microscope slides. Wholemounts were incubated with rabbit polyclonal anti–vasoactive intestinal polypeptide (VIP) (1:1,000; Diastorin, Stillwater, MN) or rabbit polyclonal anti–calcitonin gene-related peptide (CGRP) (1:500; Affiniti Research Products, Manhhead, U.K.) for 15 h at room temperature. After washing, preparations were incubated with FITC–conjugated goat anti-rabbit IgG for 90 min and counterstained with pontamine sky blue as described above.

In all cases, tissue from treated and untreated groups were processed in parallel with their respective controls. Samples were examined using an EDGE 3-D head fluorescence microscope (EDGE Scientific Instrument, Santa Monica, CA) and photographed with a Canon EOS Elan II camera. Images were captured and micrographs were prepared using Adobe Photoshop 5.5 software.

**Biochemical analysis.** Samples of the right atrium and ileum were dissected and frozen in liquid nitrogen until analysis.

**Norepinephrine levels.** A pipette was inserted through the lumen of ileum segments of known length, and a score was made through the smooth muscle layers, leaving the underlying submucosa and mucosa intact. The smooth muscle layers with the myenteric plexus were removed using a cotton swab and the preparation was weighed. The norepinephrine (NA) content of the atria and myenteric plexus preparation was measured using high-performance liquid chromatography with electrochemical detection (HPLC–ECD), as reported previously (5). Samples were homogenized in 500 μl of 0.1 mol/l perchloric acid (PCA) containing 0.4 mmol/l sodium bisulphite and 12.5 μg of the internal standard, dibhydroxybenzylamine (DHBA; Aldrich, Poole, U.K.). After low-speed centrifugation, supernatants were subjected to alumina extraction. Samples (50 μl) were injected for HPLC. Separation was achieved using a Spherisorb ODS2 C18 reverse-phase column (Hichrom, Reading, U.K.) and a mobile phase of 0.1 mol/l sodium phosphate buffer (pH 5.0) containing 5 mmol/l heptane sulfonate (Aldrich, Poole, U.K.); 0.1 mmol/l EDTA, and 13% (vol/vol) methanol. Quantification was achieved using glassy carbon electrodes (guard cell, +0.3 V; detector 1, +0.23 V; detector 2 –0.39 V). Levels were measured using peak heights relative to standards of NA and DHBA. NA content was corrected for recovery using the internal standard.

**NOS activity.** Assays were carried out on smooth muscle/myenteric plexus preparations as described above. The NOS assay has been described in detail previously (20) and is based on the conversion of [3H]arginine to [3H]citrulline during NO synthesis. Tissue was homogenized in 10 mmol/l HEPES buffer (pH 7.4) containing 0.3 mol/l sucrose, 0.1 mmol/l EDTA, 1 mmol/l dithiothreitol, 10 μg/ml leupeptin, 10 μg/ml soybean trypsin inhibitor, and 2 μg/ml aprotinin to give a tissue concentration of 50 mg/ml. After centrifugation at 12,000g for 10 min, NOS activity was measured in the supernatant. Aliquots (25 μl) were incubated with 100 μl incubation mixture containing 50 mmol/l HEPES (pH 7.4), 1 mmol/l EDTA, 1 mmol/l dithiothreitol, 1 mmol/l NADPH, 1.25 mmol/l CaCl₂, 10 μg/ml calmodulin, 10 μmol/l flavin adenine dinucleotide, and 100 μmol/l tetrahydrobiopterin. The reaction was started by adding 25 μl of 10 μmol/l [3H]arginine with a specific activity of 4.2 Ci/mmol (NEN Life Science Products, Hounsdown, U.K.). After 15 min at 37°C, the reaction was stopped by adding 2 ml dioxev ion-exchange resin in water (1:1), vortex mixing, and placing the samples on ice. The supernatants were mixed with 5 ml Octiphase HiSafe 3 for liquid scintillation counting, and counts were corrected to dpm. Blanks consisted of samples incubated with 1 mmol/l L-arginine methyl ester (NOS inhibitor). Ca²⁺–dependent NOS activity was determined by the difference in activity in the presence and absence of 2 mmol/l EGTA, a Ca²⁺–chelator. All determinations were carried out in triplicate.

**Statistical analysis.** Quantitative data have been presented as means ± SE. Comparisons between groups were made using one-way ANOVA followed by Newman-Keuls test for multiple comparisons. P ≤ 0.05 was taken to indicate statistical significance.

**RESULTS**

All the diabetic rats used in the study had significantly increased blood glucose levels (UD, 33.5 ± 1.0 mmol/l, n = 18; LAD, 33.8 ± 2.84, n = 7; EPOD, 34.2 ± 3.82, n = 8). LA and EPO treatment had no effect on blood glucose levels in diabetic or control rats (UC, 10.1 ± 0.8, n = 16; LAC, 9.4 ± 1.19, n = 6; EPOC, 8.6 ± 0.94, n = 8).

**Heart.** The sympathetic innervation of the right atrium was assessed by the biochemical measurement of NA levels (Figs. 1A and B). Diabetes caused a significant reduction (P < 0.05) in NA content. LA treatment completely prevented the diabetes-induced decrease (UD vs. LAD, P < 0.001), and NA levels in the LAD group were not significantly different from either UC or LAC groups. In
contrast, EPO treatment failed to prevent the decrease in NA in diabetes and also caused a significant reduction of NA levels in controls (UC vs. EPOC, P < 0.05).

**Penis.** In the UC group, fluorescent nerve fibers containing NOS-I could be observed throughout the erectile tissue of the corpus cavernosum urethrae (Fig. 2A). In the UD group there was a dramatic reduction in NOS-I–containing nerve fibers (Fig. 2B). LA treatment prevented this diabetes-induced decrease such that the pattern of NOS-I immunoreactivity in the LAD group was similar to UC rats (Fig. 2D). Surprisingly, LA treatment of controls resulted in a consistent decrease in NOS-I–containing nerve fibers relative to UCs (Fig. 2C). EPO treatment failed to prevent the diabetes-induced decrease in NOS-I–containing nerve fibers (Fig. 2F) and had no effect on controls (Fig. 2E).

VChT is a marker for parasympathetic nerves containing acetylcholine (21). No difference was observed in VChT-containing nerve fibers in any of the groups studied, indicating that there had been no loss of parasympathetic nerve fibers at this stage of diabetes (data not given).

**Ileum**

NA-containing nerves. The extrinsic sympathetic innervation of the ileum was assessed by the distribution of TH immunoreactivity and measurement of NA content. TH immunoreactivity could be observed in nerve fibers in the myenteric ganglia and within the smooth muscle, with considerable variability between preparations (data not given). Therefore, assessment of the treatments was carried out by quantitative analysis of NA content (Figs. 3A and B). There was a tendency for the weight per centimeter of the smooth muscle/myenteric plexus preparations to be increased in the diabetic groups. This has been reported before and is due to smooth muscle hypertrophy. To prevent the presence of hypertrophy influencing the data, results have been calculated as per centimeter of ileum rather than as gram of wet weight tissue (4, 22). Diabetes resulted in a significant reduction (P < 0.01) of the NA content of the ileum. Neither LA nor EPO treatment prevented this diabetes-induced decrease in NA. LA and EPO treatment did not significantly affect the NA content of controls (Figs. 3A and B).

**NOS-I–containing nerves.** In UC rats, NOS-I immunoreactivity was observed in neuronal cell bodies within the myenteric ganglia and in nerve fibers within the myenteric plexus and smooth muscle layers (Fig. 4A). In the UD group there was an increase in fluorescence intensity, particularly in the nerve fibers of the circular muscle layer, which also appeared thickened (Fig. 4B). It was difficult to detect consistent patterns of change in the treated groups, although the NOS-I–containing nerve fibers in the circular muscle layer from the EPOD group resembled those of controls more closely than those of the UD group (Fig. 4F).

The results for soluble Ca^{2+}-dependent NOS activity, representative of neuronal NOS-I, have been given in Fig. 5A and B. The fact that the activity was Ca^{2+}-dependent excludes type II NOS activity, which is Ca^{2+}-independent. In addition, under the conditions used for the assay, it has been reported previously that type III NOS, present in endothelial cells, does not contribute significantly to activity (20). Diabetes resulted in a significant increase (UC vs. UD, P < 0.05) in NOS-I activity in the smooth muscle/myenteric plexus preparation. LA treatment partially pre-
vent the increase induced by diabetes such that values for the LAD group were between those of UD and UC. NOS-I activity tended to be lower in the LAC group but not significantly. EPO treatment completely prevented the diabetes-induced increase in NOS-I activity, the NOS-I activity for the EPOD group being significantly lower (P < 0.05) than the UD group. EPO treatment resulted in a nonsignificant tendency for increased NOS-I activity in controls.

VIP-containing nerves. In UC preparations, VIP immunoreactivity could be observed in a dense meshwork of nerve fibers within the myenteric ganglia and the smooth muscle layers. Positive neuronal cell bodies staining for VIP were often observed in control preparations but could readily be demonstrated in the UD group (Figs. 6A and B). Neither LA nor EPO treatment had any observable effect on control or diabetic preparations (Fig. 6C–F).

The frequency of positive staining for VIP in neuronal cell bodies was quantified (Figs. 7A and B). The percentage of neuronal cell bodies positive for VIP was significantly greater in the UD group than in the UC group (P < 0.001). Neither LA nor EPO treatment significantly affected control values or prevented the diabetes-induced increase in VIP-containing cell bodies.

CGRP-containing nerves. Representative micrographs demonstrating CGRP-containing nerves in wholemount preparations of the myenteric plexus of the ileum have been given in Fig. 8A–F. In UC rats, CGRP immunoreactivity could be observed in a meshwork of fibers surrounding the neurons in the myenteric ganglia. In addition, varicose CGRP-containing nerve fibers were observed within the ganglia and smooth muscle (Fig. 8A). Untreated diabetes resulted in a marked decrease in the meshwork of CGRP-containing nerve fibers in the ganglia. CGRP immunoreactivity was largely confined to single, highly varicose nerve fibers (Fig. 8B). Neither LA nor EPO had any effect on CGRP immunoreactivity in control or diabetic ileum (Fig. 8C–F).

DISCUSSION

Oxidative stress occurs in diabetes by a variety of mechanisms including auto-oxidation of glucose, production of advanced glycation end products, and reductions in antioxidant defense mechanisms caused by activation of the polyol pathway (9,10). Since vitamin E deficiency or prooxidant treatment causes peripheral nerve deficits similar to those in streptozotocin-diabetic rats, it has been proposed that oxidative stress plays an important role in the development of diabetic neuropathy (23–25). Indicators of oxidative stress are increased in peripheral nerve trunks and sympathetic superior cervical ganglia from streptozotocin-diabetic rats (24). Considerable interest has been shown for using LA in antioxidant treatment of diabetes complications since it acts in both aqueous and lipid environments (26). LA prevents nerve conduction velocity deficits in streptozotocin-diabetic rats (11). Further, LA has undergone clinical trials for the treatment of diabetic neuropathy with reported beneficial effects (27).
Another metabolic defect that is known to occur in diabetes is reduced synthesis of \(\gamma\)-linolenic acid, and this can contribute to diabetes complications by causing changes in membrane structure and abnormal eicosanoid production (6). Treatment with \(\gamma\)-linolenic acid prevents nerve conduction deficits in streptozotocin-diabetic rats (14,15) and improves peripheral nerve function in humans (28). However, less is known about the effects of these treatments on autonomic neuropathy in diabetes. The results of the present study confirm that a variety of changes occur in autonomic nerves in diabetes and demonstrate that LA treatment was more effective in preventing these diabetes-induced changes than EPO. In addition, the effectiveness of treatment was shown to vary with the target organ studied.

In the heart, diabetes resulted in a small but significant reduction in NA levels that was totally prevented by LA treatment. It has been reported that treatment with the antioxidant vitamin E prevents the loss of catecholamine fluorescence in nerve fibers in the heart in streptozotocin-induced diabetes (29). Sympathetic denervation has also been demonstrated in the left ventricle of the heart of humans with diabetes (30). Furthermore, beneficial effects of LA treatment on cardiac autonomic neuropathy have been reported in a clinical trial (31). In contrast, EPO was without any preventative effect in the heart and actually caused a decrease in NA levels in controls. At this stage we cannot conclude whether the decrease in control NA levels following EPO treatment was due to an effect of \(\gamma\)-linolenic acid per se or due to the increased oil content of the diet when using EPO as the source.

In the penis, NO synthesized by NOS-I is the major neurotransmitter involved in parasympathetic nerve-mediated relaxation of erectile tissue (32). The major pelvic ganglion in the rat is the source of NOS-containing nerve fibers in the penis (33) where NOS-I is colocalized with acetylcholine and VAChT in parasympathetic nerves (21). Downregulation of NOS-I protein and mRNA expression occurs in erectile tissue from streptozotocin-diabetic rats (34). In addition, a reduction in neurogenic relaxation (dependent on NO) has been reported in erectile tissue in streptozotocin-diabetic rats (35,36) and in humans with diabetes, and has been implicated in the development of impotence in diabetic males (37,38). In the present study, diabetes caused a marked decrease in NOS immunoreactivity in the corpus cavernosum urethrae, which was prevented by LA treatment but not EPO. Although degeneration of NOS-containing nerve fibers has been reported in erectile tissue in streptozotocin-diabetic rats (35,36), we did not find evidence of nerve fiber loss. The pattern of staining with the parasympathetic nerve marker, VAChT, was unchanged in erectile tissue from untreated diabetic rats. Thus, the decrease observed in NOS immunoreactivity reflected a decrease in expression of NOS-I rather than loss of nerve fibers. Surprisingly, LA treatment caused decreased expression of NOS-I in nerve fibers in controls. Nitric oxide is a free radical and its actions as a neurotransmitter can be prevented by reactive oxygen species (ROS) (39). It is possible that LA treatment of controls reduced the baseline level of production of ROS, such that the basal rate of synthesis of NO could be reduced, and
this was reflected in reduced expression of NOS-I. NOS-I activity also tended to be lower in the ileum in LA-treated controls, though not significantly. In a pharmacological study of erectile tissue from streptozotocin-diabetic rats, a deficit in nerve-mediated relaxation occurred that was prevented by LA treatment (35). No change in the responses of erectile tissue from control rats receiving LA treatment was observed. Thus, it remains to be determined whether the decrease in NOS-I expression reported here, in controls receiving LA, has functional consequences.

The diabetes-induced changes in NA-, NOS-, VIP-, and CGRP-containing nerves supplying the ileum were similar to those reported previously (22,40,41). There is evidence that VIP- and NA-containing nerves are degenerating at this stage, whereas changes in CGRP-containing nerves reflect loss of neurotransmitter expression rather than nerve fiber loss (4,5,42,43). The degenerative process involves initial accumulation of transmitter in the cell body due to impaired release with loss of terminal nerve fibers. In contrast to both the heart and penis, LA treatment was unable to prevent any of the diabetes-induced changes in the ileum whether they involved neurotransmitter or nerve fiber loss. EPO treatment totally prevented the increase in NOS-I activity in the ileum from diabetic rats. This was the only autonomic nerve type investigated in the present study where EPO treatment had a significant preventative effect. Similar doses of γ-linolenic acid or EPO have been reported to prevent and reverse peripheral nerve deficits in streptozotocin-induced diabetes (14,15,44). The dose of LA used here was also based on previous studies reporting prevention of symptoms in vitamin E deficiency (18) and prevention of nephropathy and peripheral nerve deficits in streptozotocin-induced diabetes (11,45). The greater beneficial effect of LA compared with EPO, reported here, may indicate that the relative contribution of oxidative stress and abnormal fatty acid metabolism to the development of neuropathy in autonomic nerves differs from that in sensory and motor nerves.

The aim of this study was to investigate whether treatments of neuropathy are equally effective for different populations of autonomic nerves. Subpopulations of autonomic nerves were compared in the same animals receiving the same treatment. It can be concluded that a dose of LA, which was effective in preventing diabetes-induced changes in autonomic nerves supplying the heart and penis, was not effective for nerves supplying the ileum. This study does not exclude the possibility that a higher dose of LA would have also prevented diabetes-induced changes in enteric nerves. However, it is of relevance to clinical studies that it be recognized that some autonomic nerves may be more difficult to treat than others. The assessment of autonomic neuropathy in diabetes largely relies on cardiovascular function tests. It is recognized that current clinical tests for autonomic function fail to adequately assess either the extrinsic supply to the gastrointestinal tract or the integrity of the intrinsic enteric nervous system (3). In the single clinical study assessing LA treatment of autonomic neuropathy, one dose of LA was examined and only cardiac autonomic nerve function was assessed with beneficial effects being reported (31). It would be of interest to include assessment of symptoms of disordered gut motility in future clinical studies of this type.

The results presented here raise the question as to why different populations of autonomic nerves respond differently to LA treatment. Because both NA and NO are susceptible to oxidation, it could be argued that antioxidant treatment would be more effective for nerves containing these transmitters than for nerves containing the neuropeptides CGRP and VIP. However, this does not explain why diabetes-induced changes in NA- and NOS-containing nerves supplying the ileum were not. Studies of neurodegenerative disorders in the central nervous system have suggested that different populations of neurons have different capacities to defend themselves against oxidative stress, and this accounts for their relative susceptibility to neurodegeneration (46,47). Whether levels of defense also vary between different subpopulations of autonomic nerves resulting in differential vulnerability has not been established but merits investigation in light of the present findings. It is also possible that the relative metabolic activity of different autonomic nerve populations may be of significance. Using cytochrome oxidase as a marker for mitochondrial and metabolic activity, it has already been reported that subpopulations of both pelvic and myenteric neurons have markedly different levels of activity. Metabolic activity can be correlated with the functional activity of the individual neurons, i.e., their frequency of firing, under both normal...
and pathological conditions (48–50). Since mitochondria are a major site of production of ROS (46), the functional activity of different populations of autonomic neurons may well influence their relative susceptibility to oxidative stress in diabetes and to antioxidant treatment.

In conclusion, the results of the present study demonstrate that treatment with LA is more effective than EPO at preventing diabetes-induced changes in autonomic nerves supplying the heart and penis. However, neither treatment could prevent many of the changes that occur in different populations of autonomic nerves supplying the ileum. Future clinical studies investigating the effectiveness of different treatments for diabetic autonomic neuropathy will need to take into account the fact that autonomic nerves supplying different target organs may not be equally responsive to such treatment.

ACKNOWLEDGMENTS
This study was funded by the Juvenile Diabetes Research Foundation International (Grant no. 1-1999-560) whose support is gratefully acknowledged.

REFERENCES

41. Lincoln J, Messersmith WA, Belai A, Burnstock G: The effects of streptozotocin-induced diabetes and ganglioside treatment on the activities of the


