Br. J. Pharmacol. (1993), **110**, 1105–1111 © Macmillan Press Ltd, 1993

Impaired sensory-motor nerve function in the isolated mesenteric arterial bed of streptozotocin-diabetic and ganglioside-treated streptozotocin-diabetic rats

Vera Ralevic, Abebech Belai & [1]Geoffrey Burnstock

Department of Anatomy and Developmental Biology and Centre for Neuroscience, University College London, Gower Street, London WC1E 6BT

1 Adult male Wistar rats were treated with streptozotocin (65 mg kg^{-1}, i.p.) to induce diabetes. Subgroups of age-matched control and streptozotocin-treated rats were given daily injections of mixed brain bovine gangliosides (60 mg kg^{-1} body weight, i.p.). At eight weeks after treatment mesenteric arterial beds from rats in each of the four groups were isolated and perfused and the function of perivascular nerves (sympathetic and sensory-motor), endothelium and smooth muscle was assessed.

2 Values for basal tone of mesenteric beds from diabetic and diabetic-ganglioside rats were significantly lower than those of the control and control-ganglioside-treated rats. Perfusion pressures at basal tone were 25.55 ± 0.8 ($n = 11$), 22.58 ± 1.5 ($n = 12$), 28.42 ± 1.6 ($n = 12$) and 30.67 ± 1.9 ($n = 12$) mmHg for diabetic, diabetic-ganglioside, control and control-ganglioside-treated rats respectively.

3 There was no difference between the groups with respect to vasoconstrictor responses to sympathetic nerve stimulation, or to doses of noradrenaline. Vasoconstrictor responses to potassium chloride were also similar between the groups.

4 Perivascular nerve stimulation in the presence of the sympathetic blocker guanethidine (3 μM), with tone of the preparation raised with methoxamine (3–100 μM), elicited frequency-dependent vasodilatation of mesenteric arterial beds due to transmitter release from sensory-motor nerves. Sensory-motor nerve-induced vasodilator responses of mesenteric arterial beds from streptozotocin-diabetic and ganglioside-treated diabetic rats were significantly smaller than those of mesenteric beds from the controls (untreated and ganglioside-treated). Vasodilator responses to exogenously applied calcitonin gene-related peptide, the principal vasodilator transmitter released from these nerves, were not different between the groups. Vasodilator responses to the sensory neurotoxin capsaicin were also not different between the groups.

5 Endothelium-dependent vasodilator responses to acetylcholine were similar between the groups as were those to the endothelium-independent vasodilator sodium nitroprusside.

6 These results indicate that streptozotocin-induced diabetes produces marked impairment of sensory-motor nerve function in the rat mesenteric arterial bed. The significantly lower basal perfusion pressures of mesenteric beds from diabetic rats compared to controls may be a reflection of sympathetic dysfunction, but no differences were apparent from the vasoconstrictor responses produced when sympathetic nerves were electrically stimulated. There was no evidence for changes in endothelial vasodilator function, or smooth muscle vasodilator and vasoconstrictor function. Ganglioside treatment did not modify any aspect of vascular function of mesenteric beds from streptozotocin-diabetic or control rats.

Keywords: Sensory afferents; diabetes; gangliosides; mesenteric arterial bed; endothelium

Introduction

Diabetes is known to produce pathological changes in blood vessel structure and function involving both nerves (sensory, motor and autonomic) and endothelial cells. The pathogenesis of diabetic neuropathy has been extensively studied in the streptozotocin-treated rat, a model of insulin-dependent diabetes. In this model, sympathetic dysfunction is suggested by attenuated contractile responses due to stimulation of sympathetic nerves in the rat mesenteric bed (Takiguchi *et al.*, 1988) and tail artery (Hart *et al.*, 1988). Ultrastructural changes in sympathetic ganglia (Monckton & Pehowich, 1982) and neuronal deficits in tyrosine hydroxylase (the rate-limiting enzyme in the synthesis of noradrenaline) and 5-hydroxytryptamine (5-HT) specific to mesenteric perivascular sympathetic nerves (Webster *et al.*, 1991) have also been seen. The literature is conflicting with regard to the effect of diabetes on contractile activity to various agents including α-adrenoceptor agonists, 5-HT, K^+ and prostaglandins since increased vascular reactivity (Agrawal & McNeill, 1987; Abebe & MacLeod, 1990; Abebe *et al.*, 1990; White & Carrier, 1990) as well as no change (Furman & Sneddon, 1993) or diminished responsiveness (Longhurst & Head, 1985) to these same agents have been reported. Sensory-nerve conduction velocity has been reported to be decreased in diabetes (Moore *et al.*, 1980; Julu, 1988) and sensory neuropeptide-like immunoreactivity has been shown to be diminished in human diabetic skin (Levy *et al.*, 1989) and in *in vivo* corrected data in streptozotocin-diabetic rat mesenteric vessels (Webster *et al.*, 1991). Functional studies of perivascular sensory-motor nerves in diabetes, however, are relatively scarce, partly because of the few preparations in which motor responses to these nerves can be recorded. One such preparation is the isolated rat mesenteric arterial bed, in which electrical stimulation of capsaicin-sensitive primary sensory afferents during sympathetic blockade elicits vasodilatation which is mediated by the sensory neuropeptide, calcitonin gene-related peptide (CGRP) (Kawasaki *et al.*, 1988; Rubino *et al.*, 1992).

It is now known that endothelial cells play a crucial role in the control of blood vessel tone, particularly as mediators of

[1] Author for correspondence.

vasodilatation to a number of vasoactive agents (Furchgott, 1983). Several studies have claimed that endothelial cells are adversely affected by diabetes, impaired endothelium-dependent relaxations having been demonstrated in clinical (De Tejada *et al.*, 1989) and experimental (Takiguchi *et al.*, 1988; Kamata *et al.*, 1989; Mayhan, 1992; Miyata *et al.*, 1992; Taylor *et al.*, 1992) diabetes. On the other hand, supersensitivity (Gebremedhin *et al.*, 1987; White & Carrier, 1990) and no change (Fortes *et al.*, 1983; Gebremedhin *et al.*, 1987; Andersson *et al.*, 1992; Furman & Sneddon, 1993) in endothelial responses in diabetes have also been described. The function of the underlying vascular smooth muscle in diabetes is generally unimpaired.

The aim of the current study was to assess the effects of streptozotocin-induced diabetes on peripheral nerve function in the rat mesenteric arterial bed. The functions of sympathetic and sensory-motor nerves were examined, as were postjunctional responses to the sympathetic neurotransmitter noradrenaline (NA), and the sensory neurotransmitter CGRP. The function of the vascular endothelium was assessed using the endothelium-dependent vasodilator, acetylcholine (ACh), and that of the underlying vascular smooth muscle was examined with the endothelium-independent vasodilator, sodium nitroprusside (SNP). Recently there has been considerable interest in the protective role of gangliosides (glycosphingolipids found in abundance in membranes from nervous tissue) in diabetes due to reports that these have improved some aspects of experimental diabetic neuropathy (Calcutt *et al.*, 1988; Ekstrom & Tomlinson, 1990; Soediono *et al.*, 1993). In view of this, we also examined the effects of ganglioside pretreatment on aspects of mesenteric arterial function in streptozotocin-induced diabetes.

Methods

Diabetes was induced in 23 adult male Wistar rats (weighing 400–450 g) by a single intraperitoneal injection (65 mg kg^{-1} body weight) of buffered streptozotocin (Belai *et al.*, 1988). Controls ($n = 24$) consisted of untreated animals of the same initial weight range. A week after the streptozotocin injection, subgroups of streptozotocin-treated ($n = 12$) and control (untreated) ($n = 12$) rats were given daily injections of a mixture of gangliosides (AFG_1) (60 mg kg^{-1} body weight per week; Fidia Abano, Terme, Italy). The onset of diabetes was established by the presence of rapid weight loss, polyuria and glycosuria. All groups were maintained under the same conditions, supplemented with food and water *ad libitum* until death, at 8 weeks. Previous studies in this laboratory showing no changes in a subgroup of streptozotocin-injected rats which failed to develop diabetes at any time during the 8 week period after streptozotocin injection are consistent with this model being one of streptozotocin-induced diabetes, rather than of the effects of streptozotocin *per se*.

Blood samples were taken from the posterior vena cava for blood glucose analysis under ether asphyxiation. Mesenteric arterial beds were isolated and set up for perfusion essentially as described previously (Ralevic & Burnstock, 1988). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, the gut dissected away and the preparation mounted on a stainless steel grid (7 cm × 5 cm) in a humid chamber. The preparation was perfused at a constant flow rate of 5 ml min^{-1} by use of a peristaltic pump (Cole Parmer Instruments). Perfusion was with Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2:5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mmHg) with a pressure transducer (model P23, Gould) on a side arm of the perfusion cannula, and recorded on a polygraph (model 79D, Grass). The preparation was allowed to equilibrate for 30 min prior to experimentation.

Stimulation of perivascular nerves was achieved by passing a current between the cannulation needle and the wire grid on which the preparation rested. Sympathetic nerves were activated at basal tone by electrical field stimulation (90 V, 1 ms, 4–32 Hz for 30 s), and the resulting vasoconstriction could be abolished by guanethidine treatment. In separate preparations guanethidine (3 μM) was added to the perfusate at basal tone after 20 min equilibration and was present in the perfusate thereafter; the effectiveness of this treatment was confirmed after 10 min by establishing that vasoconstrictor responses to stimulation of sympathetic nerves were abolished. The tone of the preparation was then raised by the addition of methoxamine to the perfusate to a final concentration of 3–300 μM. Transmural nerve stimulation (60 V, 0.1 ms, 1–12 Hz, for 30 s) elicited vasodilator responses due to activation of primary sensory afferents and subsequent release of sensory transmitter. These vasodilator responses could be abolished by capsaicin confirming their sensory origin.

Drugs were administered as 50 μl bolus injections via an injection port proximal to the tissue. Vasoconstrictor responses to increasing doses of NA and ATP were established at basal tone at 3 min intervals or after tone had returned to baseline. Vasodilator responses to increasing doses of ACh, CGRP and SNP were established after the tone of the preparation had been raised with methoxamine. This was followed by a single dose of capsaicin (50 pmol). Intervals between doses were determined by the time it took for tone to return to its preconstricted level. Constrictor responses to potassium chloride (KCl, 0.15 mmol), at basal tone (following washout of methoxamine), were established at the end of each experiment as a measure of the contractile potential of the vascular smooth muscle.

Drugs

Acetylcholine chloride, sodium nitroprusside, noradrenaline bitartrate, methoxamine hydrochloride and capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) were obtained from Sigma, Poole, Dorset. Calcitonin gene-related peptide was from CRB Ltd., Cambridge. All drugs were made up in distilled water, except for noradrenaline, which was made up as a 10 mM stock solution in 0.1 mM ascorbic acid. Streptozotocin was donated by the Division of Cancer Treatment, National Institutes of Health, Bethesda, MD, U.S.A. Gangliosides were from Fidia Research Laboratories, Albano Terme, Italy.

Data analysis

All results were expressed as the mean ± s.e. Data analysis was done by analysis of variance, followed by Tukey's test to see where the differences lie. $P < 0.05$ was taken as significant.

Results

Diabetic model

The untreated controls gained weight during the 8 week period, reaching a final body weight of 566 ± 19.8 g ($n = 6$). Ganglioside-treated control rats weighed 611 ± 13.2 g ($n = 6$). Both the diabetic rats and the diabetic rats treated with gangliosides lost weight to a similar extent. Final body weight of the diabetic rats was 386.8 ± 27.1 g ($n = 6$) and of ganglioside-treated diabetic rats was 397.3 ± 16.3 g ($n = 6$). All diabetic rats used in the present study were severely hyperglycaemic with blood glucose levels of 43.4 ± 3.7 mmol l^{-1} (diabetics, $n = 6$) and 51.07 ± 2.9 mmol l^{-1} (ganglioside-treated diabetics, $n = 6$). Blood glucose levels of control rats

were 9.93 ± 0.7 mmol l^{-1} (controls, $n = 6$) and 12.17 ± 0.6 mmol l^{-1} (ganglioside-treated controls, $n = 6$). Distension of the large and small intestines and the presence of pale watery stools were observed at the time of death as previously described (Belai & Burnstock, 1990).

Basal tone

Diabetic and ganglioside-treated diabetic rats had significantly lower basal perfusion pressures than the control and ganglioside-treated control groups. Basal perfusion pressures were : 28.42 ± 1.6 ($n = 12$), 30.67 ± 1.9 ($n = 12$), 25.55 ± 0.8 ($n = 11$) and 22.58 ± 1.5 ($n = 12$) mmHg in mesenteric beds from control, ganglioside-control, diabetic and ganglioside-diabetic rats respectively. There was no significant difference between the groups with respect to vasoconstrictor responses to 0.15 mmol KCl. Values obtained were: controls, 55.75 ± 7.8 mmHg ($n = 12$); ganglioside-treated controls, 60.09 ± 7.9 ($n = 11$); diabetics, 53.91 ± 7.4 ($n = 11$); ganglioside-treated diabetics, 62 ± 6.3 ($n = 12$).

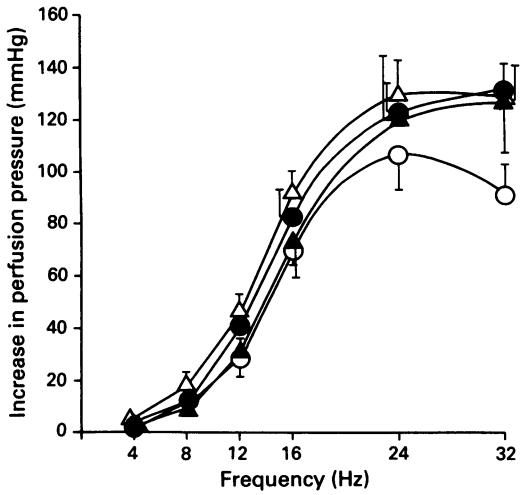

Figure 1 Frequency-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to electrical field stimulation of sympathetic nerves (4–32 Hz, supramaximal voltage, 1 ms, for 30 s): (●) control ($n = 6$); (▲) ganglioside-control ($n = 6$); (○) streptozotocin-diabetic ($n = 6$); (△) ganglioside-treated streptozotocin-diabetic ($n = 5$).

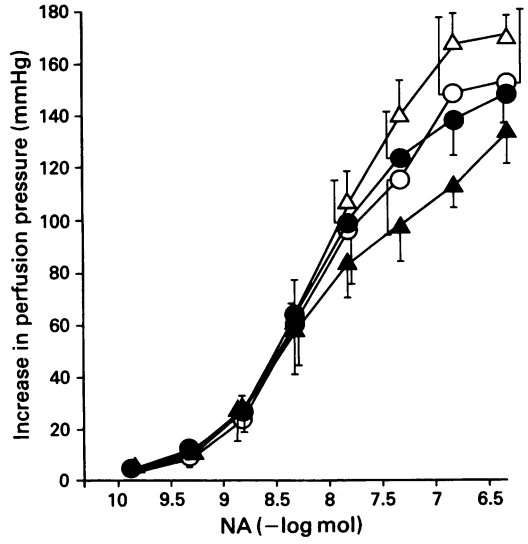

Figure 2 Dose-response curves showing vasoconstrictor responses (increase in perfusion pressure, mmHg) of rat mesenteric arterial beds to exogenous noradrenaline (NA): (●) control ($n = 6$); (▲) ganglioside-control ($n = 6$); (○) streptozotocin-diabetic ($n = 6$); (△) ganglioside-treated streptozotocin-diabetic ($n = 6$).

Sympathetic nerve stimulation

Stimulation of sympathetic nerves at basal tone elicited vasoconstrictor responses of the mesenteric vascular beds. There was no significant difference in vasoconstrictor responses between the groups (Figure 1).

Responses to noradrenaline

Mesenteric vasoconstrictor responses to NA were not different between the groups (Figure 2).

Raised-tone

There was no difference in the amount of tone produced by methoxamine in the raised-tone preparations, or in the concentration of methoxamine required to produce this degree of tone. Values for raised tone were: control, 65.65 ± 5.5 mmHg ($n = 12$); ganglioside-treated controls, 61.07 ± 6.7 mmHg ($n = 11$); diabetics, 55.4 ± 6.0 mmHg ($n = 11$); ganglioside-pretreated diabetics, 60.05 ± 5.8 mmHg ($n = 12$).

Sensory-motor nerve stimulation

Electrical field stimulation of mesenteric beds in the presence of guanethidine and methoxamine elicited frequency-dependent vasodilatation. Mesenteric beds from diabetic and ganglioside-treated diabetic rats produced significantly smaller vasodilator responses than did preparations from control and ganglioside-treated groups at 2, 4 and 8 Hz (Figures 3 and 4).

Responses to calcitonin gene-related peptide and capsaicin

There was no significant difference in vasodilator responses to CGRP or to capsaicin between the groups (Figure 5).

Responses to acetylcholine and sodium nitroprusside

There were no significant differences in vasodilator responses to ACh (Figure 6) or to SNP (Figure 7) between the groups.

Discussion

Diabetic neuropathy typically involves detrimental changes in autonomic, sensory and motor nerves. Defective axonal transport is believed to be the critical initiating factor in degenerative distal neuropathies. The present study provides a functional correlate for morphological and electrophysiological evidence for peripheral diabetic sensory neuropathy by showing that the vasodilator function of sensory-motor nerves in rat mesenteric arteries is severely impaired in streptozotocin-induced diabetes. Interestingly, we have shown in a previous study that chronic acrylamide treatment, suggested to produce similar peripheral neuropathies to diabetes, was also associated with impaired sensory-motor nerve function in the rat mesenteric arterial bed (Ralevic *et al.*, 1991).

The principal transmitter associated with the motor (efferent) function of sensory-motor nerves in the rat mesenteric arterial bed is CGRP (Kawasaki *et al.*, 1988). In the present study, while marked attenuation of sensory-motor nerve-mediated vasodilatation was observed, vasodilator responses to exogenous CGRP were not significantly different betweent the groups, indicating that streptozotocin-induced diabetes had caused peripheral sensory neuropathy and not a dysfunction of the postjunctional receptor for CGRP. There was no differences between the groups with respect to vasodilator responses to the sensory neurotoxin, capsaicin, used at a dose producing vasodilator responses of similar magnitude to those produced by electrical field stimulation at 8 Hz. Electrical stimulation of primary sensory afferents pro-

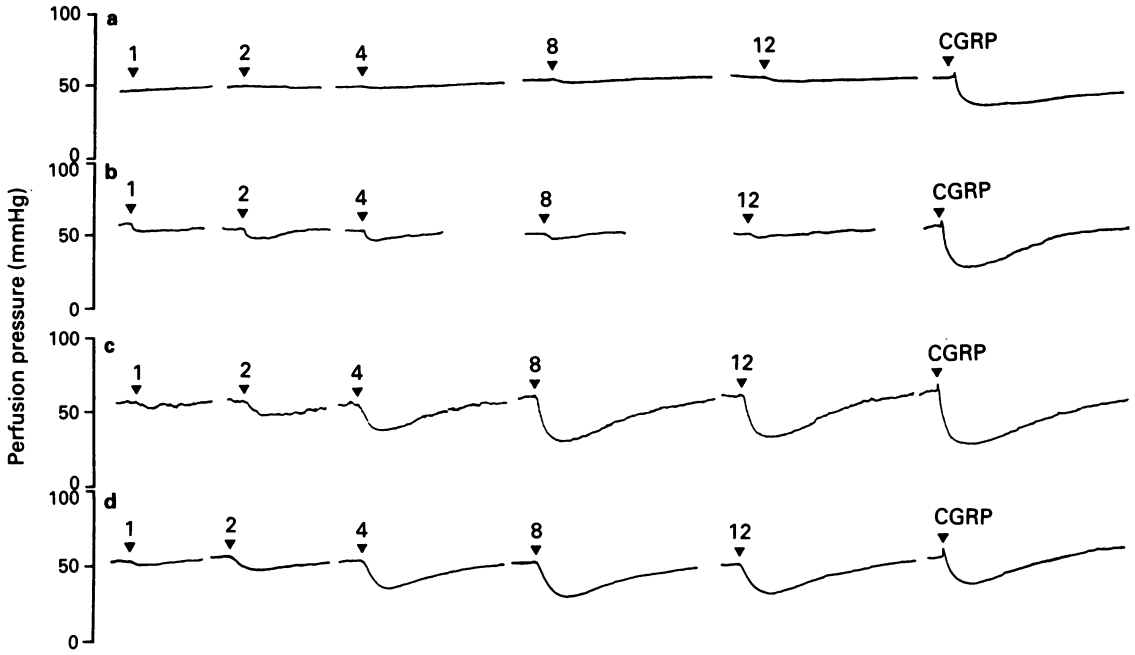

Figure 3 Traces showing frequency-dependent vasodilator responses of rat mesenteric arterial beds to electrical field stimulation of sensory-motor nerves (1–12 Hz, 60 V, 0.1 ms, for 30 s) in the presence of guanethidine (3 μM) and methoxamine. Similar vasodilator responses to a bolus dose of exogenous calcitonin gene-related peptide (CGRP, 50 pmol) were produced between the groups. Mesenteric beds from: (a) streptozotocin-diabetic; (b) ganglioside-treated streptozotocin-diabetic; (c) control; (d) ganglioside-treated control.

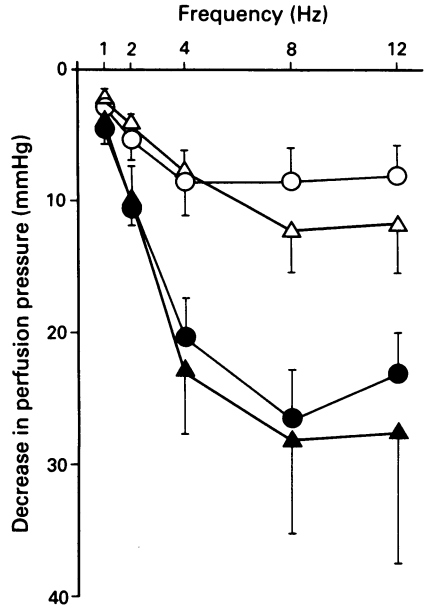

Figure 4 Frequency-response curves showing vasodilator responses of mesenteric arterial beds from control (●, $n = 6$); ganglioside-treated control (▲, $n = 5$); streptozotocin-diabetic (○, $n = 6$); ganglioside-treated streptozotocin-diabetic (△, $n = 5$) rats to sensory-motor nerve stimulation (1–12 Hz, 60 V, 0.1 ms, for 30 s) in the presence of guanethidine and methoxamine.

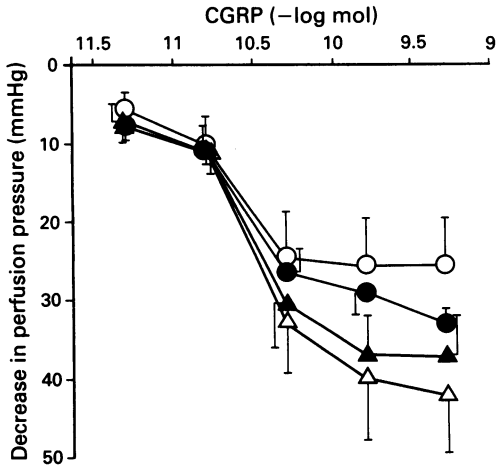

Figure 5 Dose-response curves showing vasodilator responses of rat mesenteric arterial beds to exogenous calcitonin gene-related peptide (CGRP). Control (●, $n = 6$); ganglioside-treated control (▲, $n = 5$); streptozotocin-diabetic (○, $n = 5$); ganglioside-treated streptozotocin-diabetic (△, $n = 6$).

duces antidromic invasion of sensory nerve terminals thus mimicking the axon reflex, while capsaicin stimulates the terminals of these nerves via specific receptors coupled to non-selective cation channels (Maggi & Meli, 1988). The different responses of mesenteric sensory-motor nerves to electrical stimulation, but similar responses to capsaicin which were evident between streptozotocin-diabetic and control rats indicates that the sensory defect produced by streptozotocin-diabetes is related to mechanisms which can be differentiated by physiological and non-physiological release processes.

There has been some interest in the neuroprotective role of gangliosides in diabetes following demonstrations that they are associated with prevention of the development of several neurological defects associated with short-term experimental diabetes, including axonal transport of 6-phosphofructokinase (Calcutt *et al.*, 1988) and acetylcholinesterase (Marini *et al.*, 1986), and in addition are involved in nerve regeneration (Triban *et al.*, 1989; Ekstrom & Tomlinson, 1990). On the other hand, ganglioside treatment was found not to improve impaired axonal transport of substance P in the rat sciatic nerve (Calcutt *et al.*, 1990). The mechanisms of action of gangliosides are not clearly understood but this latter study indicates that their effects are selectively beneficial

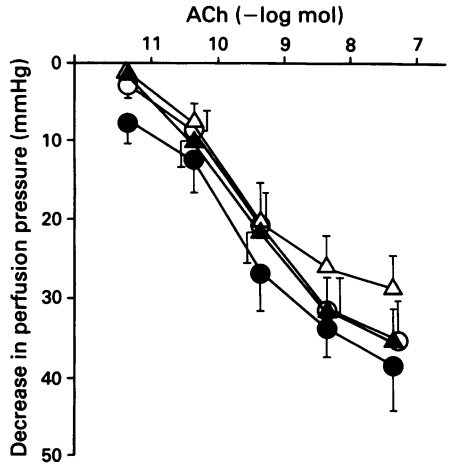

Figure 6 Dose-response curves showing vasodilator responses of rat mesenteric arterial beds to exogenous acetylcholine (ACh). Control (●, $n = 5$); ganglioside-treated control (▲, $n = 4$); streptozotocin-diabetic (○, $n = 6$); ganglioside-treated streptozotocin-diabetic (△, $n = 6$).

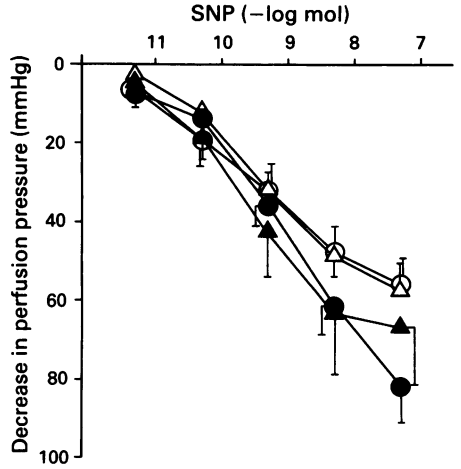

Figure 7 Dose-response curves showing vasodilator responses of rat mesenteric arterial beds to exogenous sodium nitroprusside (SNP). Control (●, $n = 6$); ganglioside-treated control (▲, $n = 6$); streptozotocin-diabetic (○, $n = 6$); ganglioside-treated streptozotocin-diabetic (△, $n = 6$).

and may explain why, in the present study, impaired mesenteric sensory-motor nerve-mediated vasodilator responses were not improved by ganglioside treatment.

The effects of experimental diabetes on sympathetic nerves, forming the principal vasoconstrictor nerve supply to mesenteric blood vessels, have been more extensively studied than have its effects on sensory-motor nerves. Other workers, also using the *in vitro* rat mesenteric arterial bed preparation, have shown that sympathetic neuropathy is a consequence of streptozotocin-induced diabetes (Longhurst & Head, 1985; Takiguchi *et al.*, 1988). In these and our study rats were used at a similar time after induction of diabetes; however, in the former studies diabetes was induced in rats weighing 200–275 g, while in our study they were 400–450 g. It is possible that the age of induction of diabetes is critically related to the susceptibility and hence to the severity of diabetes. An implication of this is that sensory-motor nerves are more susceptible to the effects of diabetes than are sympathetic nerves, thus sensory-motor dysfunction may represent an early stage in diabetic mesenteric neuropathy.

The significant decrease in basal tone of mesenteric beds from streptozotocin-induced diabetic rats seen in the present study may be a reflection of sympathetic neuropathy. However, we found that vasoconstrictor responses to electrical stimulation of sympathetic nerves were preserved. It is possible that stimulation at parameters necessary to elicit sympathetic vasoconstrictor responses may overcome subtle differences in the content and/or release of transmitter from sympathetic nerves. An alternative explanation for this apparent discrepancy involves the dynamic balance between vasoconstrictor sympathetic nerves and vasodilator sensory-motor nerves; transmural stimulation of the mesenteric bed produces responses which are the result of activation of both of these types of nerves. Hence, it is possible that sympathetic neuropathy was masked by a corresponding sensory neuropathy. On the other hand, the difference in basal tone may be unrelated to sympathetic neuropathy *per se*, but may reflect an adaptive increase in vessel calibre occurring as a consequence of the hyperphagia and marked increase in mesenteric blood flow characteristic of streptozotocin-induced diabetes. In this respect it has been reported that an enlargement of mesenteric nerves and arteries accompanies hypertrophy of the rat gut (Cracco & Gabella, 1991).

It is unlikely that the observed decrease in basal tone of the diabetic preparations seen in the present study is due to changes in the smooth muscle since vasoconstrictor responses to exogenous NA and KCl were unchanged. While this is in agreement with a recent study of the isolated mesenteric arterial bed by Furman & Sneddon (1993), the literature is variable regarding the effects of diabetes on rat mesenteric vascular responses to exogenous agents including NA and KCl, with increased (Agrawal & McNeil, 1987; Abebe & MacLeod, 1990; Abebe *et al.*, 1990; White & Carrier, 1990) or decreased (Longhurst & Head, 1985; Takiguchi *et al.*, 1988; Andersson *et al.*, 1992) contractile responses also having been shown. Some of the variability characteristic of the literature on the effects of diabetes on vascular function is likely to be due to differences in the duration of diabetes and in the different susceptibility of different vascular beds. However, it is difficult to understand why such discrepancies are also commonly reported between different groups using the same vessel and similar methods and time of induction of diabetes. The reason for these discrepancies remains unexplained.

In the present study there was no evidence for dysfunction of endothelial or smooth muscle vasodilator mechanisms. With respect to the effects of vasoconstrictor and vasodilator (endothelium-dependent and -independent) agents our results are similar to those of Furman & Sneddon (1993) who also found no evidence for changes in vascular responsiveness of the isolated rat mesenteric arterial bed after long-term (15–17 weeks) streptozotocin-induced diabetes. Furthermore, these results are compatible with those of a recent *in vivo* study by Kiff *et al.* (1991) who demonstrated raised mesenteric vascular conductance in streptozotocin-diabetic rats and no differences in endothelium-dependent vasodilatation to ACh. Attenuated relaxations to endothelium-dependent vasodilators, but generally unimpaired endothelium-independent relaxations have been demonstrated in clinical (De Tejada *et al.*, 1989) and a variety of experimental (Takiguchi *et al.*, 1988; Kamata *et al.*, 1989; Mayhan, 1992; Miyata *et al.*, 1992; Taylor *et al.*, 1992) diabetes, while endothelium-independent relaxations are generally unimpaired. It is possible that diabetes affects the different components of the vasculature with a different time course, with neuropathy occurring before the onset of endothelial changes. In this respect, Takiguchi and coworkers (1988) showed that sympathetic neuropathy in streptozotocin-induced diabetes (seen at 8 weeks after injection with streptozotocin) occurred before functional evidence for endothelial damage (occurring at 12 weeks).

In conclusion, the results of the present study provide evidence that sensory-motor nerve vasodilator function is impaired in mesenteric arteries of streptozotocin-diabetic rats. There was no evidence for a change in the postjunc-

tional receptor for CGRP. The significantly lower basal perfusion pressure shown by mesenteric beds from diabetic rats may be indicative of sympathetic neuropathy, but there was no evidence for differences in vasoconstrictor responses to electrical stimulation of sympathetic nerves. Chronic streptozotocin-induced diabetes did not modify vasoconstrictor responses to NA or KCl. Moreover, neither endothelium-dependent or endothelium-independent vasodilator responses were altered compared to age-matched controls. Ganglioside treatment did not modify any aspect of vascular function in our model. These results suggest that sensory-motor nerves may be more susceptible to diabetic neuropathy than sympathetic perivascular nerves, and these changes may precede other changes commonly reported in diabetes such as changes in the vascular endothelium.

V.R. was supported by the British Heart Foundation. The authors would like to thank Dr J. Lincoln for comments on this manuscript.

References

ABEBE, W. & MACLEOD, K.M. (1990). Protein kinase C-mediated contractile responses of arteries from diabetic rats. *Br. J. Pharmacol.*, **101**, 465–471.

ABEBE, W., HARRIS, K.H. & MACLEOD, K.M. (1990). Enhanced contractile responses of arteries from diabetic rats to α_1-adrenoceptor stimulation in the absence and presence of extracellular calcium. *J. Cardiovasc. Pharmacol.*, **16**, 239–248.

AGRAWAL, D.K. & MCNEILL, J.H. (1987). Vascular responses to agonists in rat mesenteric artery from diabetic rats. *Can. J. Physiol. Pharmacol.*, **65**, 1484–1490.

ANDERSSON, D., BRUNKWALL, J., BERQVIST, D. & EDVINSSON, L. (1992). Diminished contractile responses to neuropeptide Y of arteries from diabetic rabbits. *J. Auton. Nerv. Syst.*, **37**, 215–222.

BELAI, A. & BURNSTOCK, G. (1990). Changes in adrenergic and peptidergic nerves in the submucous plexus of streptozotocin-diabetic rat ileum. *Gastroenterology*, **98**, 1427–1436.

BELAI, A., LINCOLN, J., MILNER, P. & BURNSTOCK, G. (1988). Progressive changes in adrenergic, serotonergic, and peptidergic nerves in proximal colon of streptozotocin-diabetic rats. *Gastroenterology*, **95**, 1234–1241.

CALCUTT, N.A., TOMLINSON, D.R. & WILLARS, G.B. (1988). Ganglioside treatment of streptozotocin-diabetic rats prevents defective axonal transport of 6-phosphofructokinase activity. *J. Neurochem.*, **50**, 1478–1483.

CALCUTT, N.A., TOMLINSON, D.R., WILLARS, G.B. & KEEN, P. (1990). Axonal transport of substance P-like immunoreactivity in ganglioside-treated diabetic rats. *J. Neurol. Sci.*, **96**, 283–291.

CRACCO, C. & GABELLA, G. (1991). Enlargement of the mesenteric nerves and arteries supplying the hypertrophic ileum of the rat. *J. Anat.*, **176**, 263.

DE TEJADA, I.S., GOLDSTEIN, I., AZADOZOI, K., KRANE, R.J. & COHEN, R.A. (1989). Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. *N. Engl. J. Med.*, **320**, 1025–1030.

EKSTROM, P.A.R. & TOMLINSON, D.R. (1990). Impaired nerve regeneration in streptozotocin-diabetic rats is improved by treatment with gangliosides. *Exp. Neurol.*, **109**, 200–203.

FORTES, Z.B., LEME, J.G. & SCIVOLETTO, R. (1983). Vascular reactivity in diabetes mellitus: role of the endothelial cell. *Br. J. Pharmacol.*, **79**, 771–781.

FURCHGOTT, R.F. (1983). Role of endothelium in responses of vascular smooth muscle. *Circ. Res.*, **53**, 557–573.

FURMAN, B.L. & SNEDDON, P. (1993). Endothelium-dependent vasodilator responses of the isolated mesenteric bed are preserved in long-term streptozotocin diabetic rats. *Eur. J. Pharmacol.*, **232**, 29–34.

GEBREMEDHIN, D., KOLTAI, M.Z., POGATSA, G., MAGYAR, K. & HADHAZY, P. (1987). Differential contractile responsiveness of femoral arteries from healthy and diabetic dogs: role of endothelium. *Arch. Int. Pharmacodyn.*, **288**, 100–108.

HART, J.L., FREAS, W., MCKENZIE, J.E. & MULDOON, S.M. (1988). Adrenergic function and contractile activity of the caudal artery of the streptozotocin diabetic rat. *J. Auton. Nerv. Syst.*, **25**, 49–57.

JULU, P.O.O. (1988). The correlation between sensory nerve conduction velocities and three metabolic indices in rats treated with streptozotocin. *Diabetologia*, **31**, 247–253.

KAMATA, K., MIYATA, N. & KASUYA, Y. (1989). Impairment of endothelium-dependent relaxation and changes in levels of cyclic GMP in aorta from streptozotocin-induced diabetic rats. *Br. J. Pharmacol.*, **97**, 614–618.

KAWASAKI, H., TAKASAKI, K., SAITO, A. & GOTO, K. (1988). Calcitonin gene-related peptide acts as a novel vasodilator transmitter in mesenteric resistance vessels of the rat. *Nature*, **335**, 164–167.

KIFF, R.J., GARDINER, S.M., COMPTON, A.M. & BENNETT, T. (1991). Selective impairment of hindquarters vasodilator responses to bradykinin in conscious Wistar rats with streptozotocin-induced diabetes mellitus. *Br. J. Pharmacol.*, **103**, 1357–1362.

LEVY, D.M., KARANTH, S.S., SPRINGALL, D.R. & POLAK, J.M. (1989). Depletion of cutaneous nerves and neuropeptides in diabetes mellitus: an immunocytochemical study. *Diabetologia*, **32**, 427–433.

LONGHURST, P.A. & HEAD, R.J. (1985). Responses of the isolated perfused mesenteric vasculature from diabetic rats: the significance of appropriate control tissues. *J. Pharmacol. Exp. Ther.*, **235**, 45–49.

MAGGI, C.A. & MELI, A. (1988). The sensory-efferent function of capsaicin-sensitive sensory neurons. *Gen. Pharmacol.*, **10**, 1–43.

MARINI, P., VITADELLO, M., BIANCHI, R., TRIBAN, C. & GORIO, A. (1986). Impaired axonal transport of acetylcholinesterase in the sciatic nerve of alloxan-diabetic rats. *Diabetologia*, **29**, 254–258.

MAYHAN, W.G. (1992). Impairment of endothelium-dependent dilatation of the basilar artery during diabetes mellitus. *Brain Res.*, **580**, 297–302.

MIYATA, N., TSUCHIDA, K., OKUYAMA, S., OTOMO, S., KAMATA, K. & KASUYA, Y. (1992). Age-related changes in endothelium-dependent relaxation in aorta from genetically diabetic WBN/Kob rats. *Am. J. Physiol.*, **262**, H1104–H1109.

MONCKTON, G. & PEHOWICH, E. (1982). The effects of intermittent insulin therapy on the autonomic neuropathy in the streptozotocin diabetic rat. *Can. J. Neurol. Sci.*, **9**, 79–84.

MOORE, S.A., PETERSON, R., FELTEN, D.L. & O'CONNOR, B.L. (1980). A quantitative comparison of motor and sensory conduction velocities in short- and long-term streptozotocin- and alloxan-diabetic rats. *J. Neurol. Sci.*, **48**, 133–152.

RALEVIC, V. & BURNSTOCK, G. (1988). Actions mediated by P_2-purinoceptor subtypes in the isolated perfused mesenteric bed of the rat. *Br. J. Pharmacol.*, **95**, 637–645.

RALEVIC, V., ABERDEEN, J.A. & BURNSTOCK, G. (1991). Acrylamide-induced autonomic neuropathy of rat mesenteric vessels: histological and pharmacological studies. *J. Auton. Nerv. Syst.*, **34**, 77–88.

RUBINO, A., RALEVIC, V. & BURNSTOCK, G. (1992). Prejunctional modulation of sensory-motor nerve mediated vasodilation of the rat mesenteric arterial bed by adenosine. *Eur. J. Pharmacol.*, **220**, 95–98.

SOEDIONO, P., BELAI, A. & BURNSTOCK, G. (1993). Prevention of neuropathy in the pyloric sphincter of streptozotocin-diabetic rats by gangliosides. *Gastroenterol.*, **104**, 1072–1082.

TAKIGUCHI, Y., SATOH, .N., HASHIMOTO, H. & NAKASHIMA, M. (1988). Changes in vascular reactivity in experimental diabetic rats: comparison with hypothyroid rats. *Blood Vessels*, **25**, 250–260.

TAYLOR, P.D., MCCARTHY, A.L., THOMAS, C.R. & POSTON, L. (1992). Endothelium-dependent relaxation and noradrenaline sensitivity in mesenteric resistance arteries of streptozotocin-induced diabetic rats. *Br. J. Pharmacol.*, **107**, 393–399.

TRIBAN, C., GUIDOLIN, D., FABRIS, M., MARINI, P., SCHIAVINATO, A., DONA, M., BORTOLAMI, C., DI GIAMBERARDINO, L. & FIORI, M.G. (1989). Ganglioside treatment and improved axonal regeneration capacity in experimental diabetic neuropathy. *Diabetes*, **38**, 1012–1022.

WEBSTER, G.J.M., PETCH, E.W.A. & COWEN, T. (1991). Streptozotocin-induced diabetes in rats causes neuronal deficits in tyrosine hydroxylase and 5-hydroxytryptamine specific to mesenteric perivascular sympathetic nerves and without loss of nerve fibres. *Exp. Neurol.*, **112**, 53–62.

WHITE, R.E. & CARRIER, G.O. (1990). Vascular contraction induced by activation of membrane calcium ion channels is enhanced in streptozotocin-diabetes. *J. Pharmacol. Exp. Ther.*, **253**, H1057–H1062.

(Received May 12, 1993
Revised June 17, 1993
Accepted June 24, 1993)