British Journal of Pharmacology (1995) 115, 373-379

Effects of endothelin receptor antagonism with bosentan on peripheral nerve function in experimental diabetes

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1 The effects of the non-selective endothelin (ET) receptor (ET_A/ET_B) antagonist, bosentan, on sciatic nerve dysfunction in experimental diabetes were investigated.

2 Rats with 5–6 weeks untreated streptozotocin-diabetes exhibited characteristic slowed motor nerve conduction velocity (mean ± s.d., 36.6 ± 3.4 m s^{-1}) and nerve laser Doppler flux (197 ± 64 arbitrary units) compared to age-matched control animals (42.7 ± 2.4 m s^{-1} and 398 ± 77 arbitrary units, respectively). Preventative treatment of diabetic rats with bosentan at 100 mg kg^{-1} day^{-1} p.o. attenuated both these deficits (39.7 ± 3.0 m s^{-1} and 305 ± 56 arbitrary units, respectively) without affecting mean arterial pressure.

3 In control and untreated diabetic rats, ET-1, 1 nmol kg^{-1} i.v., caused an initial hypotension (duration, 30 ± 13 and 26 ± 9 s, respectively; change in mean arterial pressure, −27 ± 13 and −25 ± 7 mmHg, respectively) followed by prolonged hypertension (change in mean arterial pressure, 52 ± 18 and 31 ± 5 mmHg, respectively). Effectiveness of the chronic bosentan treatment was demonstrated by inhibition of the hypotensive response to ET-1 in treated diabetic rats (duration, 5 ± 2 s; change in mean arterial pressure, −4 ± 2 mmHg) although the hypertension was unaltered (change in mean arterial pressure, 32 ± 9 mmHg).

4 Acute i.v. administration of 10 mg kg^{-1} bosentan caused variable and transient rises in nerve laser Doppler flux in control (78 ± 63 arbitrary units) and untreated diabetic rats (93 ± 77 arbitrary units). Acute bosentan blocked the hypotensive response to subsequent ET-1 administration and attenuated the later hypertension (change in mean arterial pressure, 21 ± 9 mmHg in control, 29 ± 10 mmHg in diabetic).

5 Our results indicate that oral treatment of diabetic rats with an ET receptor antagonist can improve sciatic nerve perfusion and conduction, suggesting that the vasoconstrictor action of endogenous ET may contribute to peripheral nerve dysfunction in experimental diabetes.

Keywords: Endothelin-1; bosentan; streptozotocin; diabetes mellitus; nerve conduction; nerve blood flow

Introduction

Reduced peripheral nerve conduction velocity occurs soon after development of both clinical (Gregersen, 1967) and experimental (Eliasson, 1964) diabetes. Although the aetiology of this conduction deficit is unknown, a role for nerve ischaemia is supported by findings of nerve hypoxia in diabetic patients (Newrick *et al.*, 1986) and animals (Tuck *et al.*, 1984), together with poor nerve blood flow in the latter (Tuck *et al.*, 1984; Monafo *et al.*, 1988; Yasuda *et al.*, 1989; Cameron *et al.*, 1991). Furthermore, several compounds which improve nerve conduction in experimental diabetes appear to act by attenuating nerve ischaemia (Yasuda *et al.*, 1989; Stevens *et al.*, 1993b; Maxfield *et al.*, 1993; Cameron *et al.*, 1994a). The mechanism(s) responsible for reduced nerve perfusion remain unclarified, but the disordered endothelial function associated with diabetes (Stout, 1987) may contribute. There is evidence to suggest that the release of the endothelium-derived vasodilator, prostacyclin, is impaired in peripheral nerve from diabetic rats (Ward *et al.*, 1989; Stevens *et al.*, 1993a), while treatment with its stable analogue, iloprost, ameliorates nerve dysfunction (Ohno *et al.*, 1992; Shindo *et al.*, 1992; Cotter *et al.*, 1993). It is possible that either an enhanced production of and/or response to the endothelium-derived, potent vasoconstrictor, endothelin (ET) may also participate in development of reduced nerve perfusion. Clinical studies show variable changes in plasma ET levels of diabetes (Takahashi *et al.*, 1990; Predel *et al.*, 1990), although recent reports indicate similar ET-1 levels to non-diabetic subjects with little dependence on the presence of complications (Bertello *et al.*, 1994; Patino, 1994; Gruden *et al.*, 1994). The likelihood that the conversion of big ET-1 to ET-1 is impaired (Tsunoda *et al.*, 1991) may have masked results from earlier studies with less specific assays. Animal studies also report contradictory change in ET levels (Takahashi *et al.*, 1991; Takeda *et al.*, 1991), although disturbed renal function may be linked with increased ET production (Fukui *et al.*, 1993; Morabito *et al.*, 1994). There is little evidence for a diabetes-induced alteration in vascular responsiveness to ET (Kiff *et al.*, 1991b), but direct effects on nerve function have not been investigated.

Hence, the primary aim of this study was to assess the role of the vasoconstrictor, ET, in peripheral nerve dysfunction in experimental diabetes. We have previously measured sciatic nerve laser Doppler flux, as an index of blood flow, and have demonstrated reductions in streptozotocin-diabetes (Stevens *et al.*, 1993b; 1994). The effects of treatment of diabetic rats with the non-selective ET_A/ET_B receptor antagonist, bosentan (Ro 47-0203), on both sciatic nerve laser Doppler flux and motor conduction velocity were determined. In addition, we also tested the effects of intravenous bosentan administration on nerve laser Doppler flux in untreated control and diabetic rats in an acute protocol, to determine any dynamic influence of ET receptor antagonism on peripheral nerve perfusion. In all animals cardiovascular responses to ET-1 were recorded as a measure of bosentan antagonism. This non-peptide, non-selective antagonist was chosen as the actions of the ET family appear to involve both ET_A and ET_B receptors (Bigaud & Pelton, 1992; Warner *et al.*, 1993) and bosentan has been clearly shown to antagonize both central and regional vascular responses to these peptides (Clozel *et al.*, 1994; Gardiner *et al.*, 1994). In addition, a peptide antagonist

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such as BQ-123 may be inadequately selective as its antagonism of the actions of other vasoconstrictor peptides such as angiotensin II, admittedly possibly via antagonism of endothelin receptors (Webb *et al.*, 1992), cannot be excluded.

Methods

Experimental organisation

Male Wistar rats (starting weight 355–400 g and 12–14 weeks of age; Charles River (UK) Ltd., Margate, UK) were assigned at random to three groups. Animals were fasted overnight and the following morning rats of two groups were given a single intraperitoneal injection of 65 mg kg^{-1} streptozotocin. Two days later, blood samples were obtained by tail prick from the streptozotocin-injected rats and blood glucose concentrations were measured by strip-operated reflectance photometry (Reflolux II, Boehringer Mannheim, Mannheim, Germany). All animals had blood glucose concentrations greater than 15 mmol l^{-1} and were thus considered diabetic and included in the experiment.

The aim of the study was to determine the effect of treatment with an ET receptor antagonist on diabetes-induced deficits in sciatic nerve laser Doppler flux and motor conduction velocity. The control group and one diabetic group were left untreated. Immediately after confirmation of diabetes, rats of the second diabetic group received bosentan, a non-peptide, non-selective ET_A/ET_B receptor antagonist, at 100 mg kg^{-1} day^{-1} by gavage for the five to six week protocol. This dose was selected because 100 mg kg^{-1} p.o. bosentan has previously been demonstrated to inhibit pressor responses to big ET-1 for up to 24 h after a single administration in control rats (Clozel *et al.*, 1994); the dose was therefore considered appropriate for use in a once-daily protocol. Animals were studied in mixed batches and, on the day of study, treated rats were dosed 1 h prior to experiment. After death, blood samples from the carotid artery were centrifuged (9000 *g* for 3 min) to provide plasma for later spectrophotometric assay of glucose concentration (GOD-PERID test kit, Boehringer Mannheim, as above).

Nerve laser Doppler flux and cardiovascular variables

In all animals, baseline sciatic nerve laser Doppler flux and systemic arterial pressure were measured in a similar manner to that described previously (Stevens *et al.*, 1993b; 1994). In brief, anaesthesia of rats was induced by halothane and maintained by 1.5 mg ml^{-1} alphaxalone and 0.5 mg ml^{-1} alphadolone (Saffan; Pitman-Moore Ltd, Uxbridge, UK; the manufacturer's solution was diluted 1 in 6 with 0.9% (w/v) saline) infused via the jugular vein at a rate of 8 mg h^{-1}. Systemic arterial pressure and heart rate were monitored via the left carotid artery and laser Doppler flux were measured in the left sciatic nerve using a Moor Instruments (Axminster, UK) fibre optic flow probe (Type P3; tip diameter 1.5 mm) and MBF3D flow monitor. Mean values for systemic arterial pressure, heart rate and nerve laser Doppler flux over 2 min were recorded for each rat. Body temperatures of rats were maintained at 37–38°C via biofeedback by a rectal probe and homeothermic blanket, Near-nerve temperature was monitored via a thermocouple lead connected to a Comark (Rushington, UK) electronic thermometer and was maintained at 35–36°C, with use of infra-red heat when necessary.

Motor nerve conduction velocity

In subgroups of untreated control and diabetic animals and in all bosentan-treated diabetic rats, immediately after nerve laser Doppler flux measurements, left sciatic motor nerve conduction velocity was determined. Stimulation (10 V, 150 μs duration) was via needle electrodes placed at the sciatic notch and ankle. Evoked electromyograms were recorded from the interosseous/plantar muscles and differences in latency were noted. Conduction velocity was calculated by dividing the distance between the stimulating electrodes by the latency difference. For these measurements, near-nerve temperature was maintained at 37°C with infra-red heat.

ET-1 administration

After measurement of conduction velocity, intravenous ET-1 was given to test ET receptor antagonism by bosentan treatment. This was judged in terms of the blockade of pressure responses to the vasoconstrictor in bosentan-treated animals relative to the pressure responses in untreated rats. Specifically, 1 nmol kg^{-1} ET-1, in a volume of 0.5 ml kg^{-1}, was injected via the jugular vein over 24 ± 4 s (mean ± 1 s.d. for all rats). This high dose of ET-1 was selected as one which produces large depressor and pressor changes in arterial pressure but which, in turn, has been shown to be limited by prior administration of bosentan utilised in similar protocols to those performed here (Clozel *et al.*, 1994). Systemic arterial pressure was monitored prior to ET administration and for the following 60 min.

Acute bosentan study

In other subgroups of untreated control and diabetic rats, the acute effects of bosentan administration were examined. After baseline recording of nerve laser Doppler flux and arterial pressure, 10 mg kg^{-1} bosentan, at a volume of 1 ml kg^{-1}, was injected over 35 ± 11 s via the jugular vein. This dose of bosentan was chosen because it has been shown to reduce significantly both depressor and pressor arterial pressure response to ET-1 in a similar protocol (Clozel *et al.*, 1994). Five minutes later, *i.v.* ET-1 was administered to all rats, as described above, to assess antagonism by acute bosentan administration.

Statistical analysis

Data are presented as mean ± 1 s.d. Statistical analyses were carried out by one-way analysis of variance and, where the *F* ratio gave $P < 0.05$ and there was homogeneity of variances (Cochrans C test, $P > 0.05$), group means were compared using Duncan's range tests. For some data (plasma glucose, duration and maximal change in mean systemic arterial pressure during the hypotension, changes in systemic arterial pressures during the hypertension), values were transformed to natural logarithms for statistical analysis to ensure homogeneity of variances.

Materials

Streptozotocin was obtained from ICI Pharmaceuticals (Macclesfield, UK) and was freshly dissolved in 0.9% (w/v) sterile saline to give a solution of 65 mg ml^{-1} prior to injection. Bosentan (Ro 47-0203; free sulphonamide for oral administration, Clozel *et al.*, 1994; 4-tert-butyl-*N*-[6-(2-hydroxy-ethoxy)-5-(2-methoxy-phenoxy)-2,2′-bipyrimidin-4-yl]-benzene-sulphonamide) was prepared as a fresh daily suspension in 5% Arabic gum at 100 mg ml^{-1}. The sodium salt, dissolved in sterile water at 10 mg ml^{-1}, was used for i.v. administration (Clozel *et al.*, 1994). Both forms of bosentan were kindly supplied from F. Hoffman-La Roche Ltd., Basel, Switzerland. ET-1 was obtained from Peptide Institute Inc. (Osaka, Japan) via Scientific Marketing Associates (Barnet, UK) and was dissolved in sterile physiological saline containing 1% bovine serum albumin to give a solution of 2 nmol ml^{-1}.

Results

Animals

Throughout the study, control animals each gained weight, while all diabetic rats lost weight (Table 1). At the end of the protocol, diabetic rats had significantly reduced body weights compared to control animals and were hyperglycaemic. Treatment with bosentan had no effect on these diabetes-induced changes.

Motor nerve conduction velocity

Untreated diabetic animals had significantly reduced sciatic motor nerve conduction velocity (36.6 ± 3.4 m s^{-1}; Figure 1) compared to control animals (42.7 ± 2.4 m s^{-1}; $P<0.01$). This deficit was attenuated in diabetic rats which were treated with bosentan (39.7 ± 3.0 m s^{-1}; not significantly different from control or untreated diabetic values).

Sciatic nerve laser Doppler flux and cardiovascular variables -baseline values

Sciatic nerve laser Doppler flux in untreated diabetic rats (197 ± 64 arbitrary units; Figure 1) was 49% of that for control animals (398 ± 77 arbitrary units) and was significantly ($P<0.01$) different from that for the control group. Values for bosentan-treated diabetic rats (298 ± 55 arbitrary units) were significantly ($P<0.01$) different from those of both the untreated control and diabetic groups. Systolic, diastolic mean arterial pressures, as well as heart rates, were modestly but significantly lower in untreated and bosentan-treated diabetic animals than in control rats (Table 1).

Cardiovascular variables -ET-1 administration

Intravenous administration of ET caused an initial hypotension followed by prolonged hypertension in control and untreated diabetic rats (Figure 2). In parallel to the changes in arterial pressure, there were tachycardiac followed by bradycardic responses to ET-1. Two of the control animals died during the hypertension (systolic pressures greater than 250 mmHg) and are not included in the hypertension data.

The initial hypotension, as judged by duration and maximal change in arterial pressure, was similar and not significantly different between control ($n = 6$) and diabetic ($n = 5$) rats. However, there was little evidence for depressor or tachycardic responses in bosentan-treated diabetic animals (maximal changes in arterial pressure and heart rate, $P<0.01$ *versus* control or diabetic, $n = 9$). The time from ET administration to the peak changes in arterial pressure and heart rate were similar in all rats (17 ± 4 s in control, 23 ± 16 s in untreated diabetic and 21 ± 14 s in bosentan-treated diabetic, no significant differences between groups).

The subsequent hypertension was of variable duration between all rats (note that the y-axis is in min for this section of Figure 2). The maximal changes in systemic arterial pressure were significantly ($P<0.05$) lower for untreated or bosentan-treated diabetic rats compared to those in control animals. The integrated pressure responses to i.v. ET-1 in control animals (areas under the arterial pressure curve during hypertension; 5448 ± 1062 mmHg min) were greater than those in untreated diabetic animals (3569 ± 1351 mmHg min) and responses were lower still in bosentan-treated rats (2695 ± 895 mmHg min). It was noted that blood appeared in the eyes of all the rats of the control group, yet not in those of any other animals.

Acute bosentan administration

Changes in nerve Doppler flux in rats during the acute bosentan part of the study are shown in Figure 3. Intra-

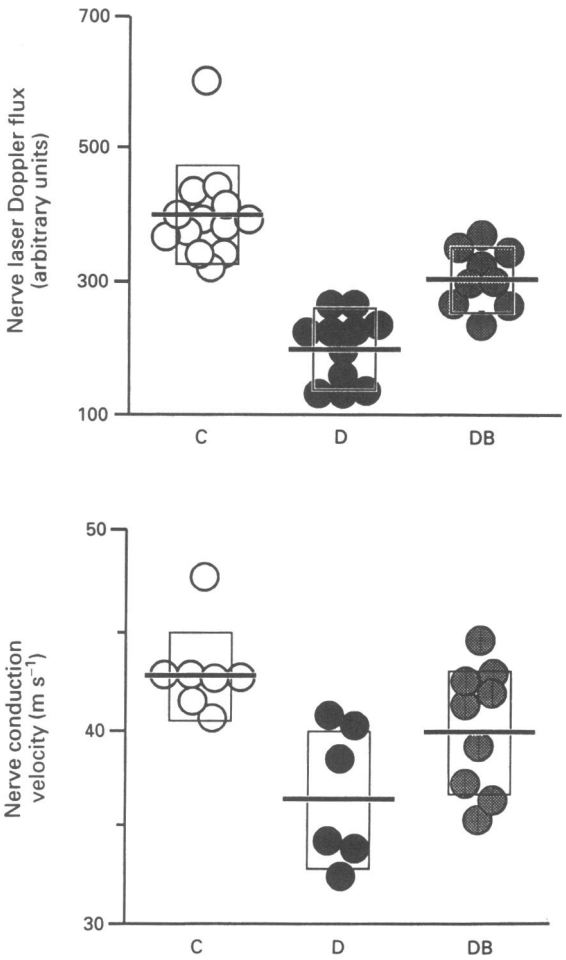

Figure 1 Point-plots showing sciatic nerve motor conduction velocity and laser Doppler flux for control rats (C; open circles), untreated diabetic rats (D; solid circles) and bosentan-treated diabetic rats (DB; cross-hatched). Individual animal data are shown with horizontal bars giving group means and boxes indicating the range of ± 1 s.d.

Table 1 Body weight, final plasma glucose, baseline systemic arterial pressure and heart rate data

	n	*Body weight* (g) *Initial*	*Final*	*Final plasma glucose* (mmol l^{-1})	*Baseline arterial pressures* (mmHg) *Systolic*	*Diastolic*	*Mean*	*Heart rate* (beats min^{-1})
Control	13	379 ± 11	544 ± 30^{x}	10.8 ± 1.9^{x}	140 ± 16^{x}	105 ± 14^{x}	119 ± 15^{x}	394 ± 35^{x}
Untreated diabetic	11	382 ± 14	311 ± 35^{y}	50.7 ± 13.2^{y}	117 ± 9^{y}	91 ± 7^{y}	104 ± 8^{y}	295 ± 39^{y}
Bosentan-treated diabetic	9	378 ± 19	286 ± 41^{y}	48.9 ± 10.3^{y}	115 ± 14^{y}	87 ± 12^{y}	100 ± 11^{y}	283 ± 32^{y}

Data are mean ± 1 s.d. and were analysed by one-way analysis of variance with Duncan's multiple range tests; $P<0.01$ (x versus y). For statistical analysis, plasma glucose data were transformed to natural logarithms to achieve homogeneity of variances

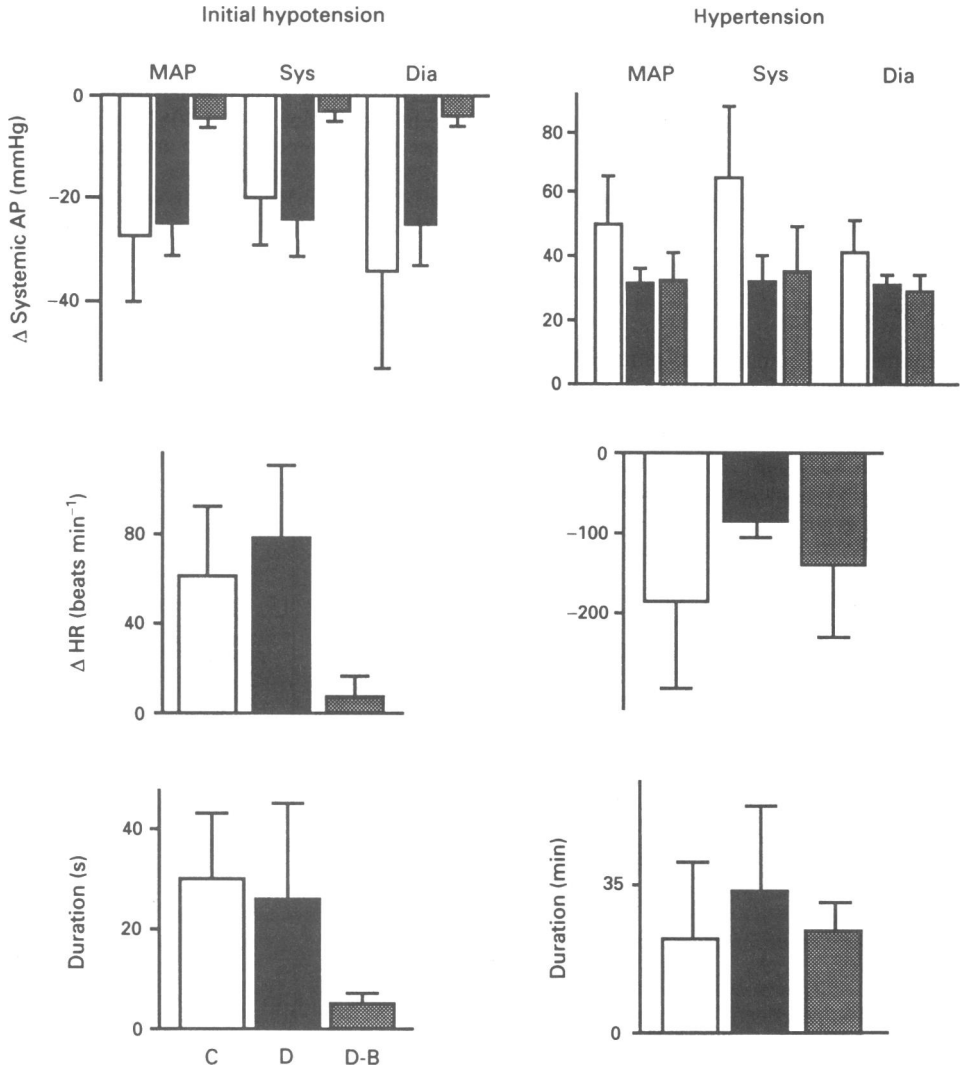

Figure 2 Maximal changes in arterial pressure (MAP = mean, Sys = systolic and Dia = diastolic) and heart rate during the initial hypotension and subsequent hypertension responses to endothelin administration, together with duration of the responses, for control rats (C; open columns), untreated diabetic rats (D; solid columns) and bosentan-treated diabetic rats (D-B; cross-hatched columns). Data are group means ± 1 s.d.

venous bosentan caused slight but brief increases in arterial pressure (maximum change; control 7 ± 5 mmHg, $n = 7$ and diabetic 20 ± 15 mmHg, $n = 6$) which were accompanied by transient decreases in heart rate (maximum change; control -17 ± 12 beats min^{-1} and diabetic -29 ± 20 beats min^{-1}). The maximal changes occurred approximately 1–2 min following the start of bosentan administration (control 90 ± 105 s and diabetic 51 ± 39 s), when there were also transient peaks in nerve Doppler flux (see Figure 3). However, the mean blood pressure or nerve Doppler flux calculated over the 5 min period (from the beginning of the administration) indicated little change from baseline (mean change in arterial pressure 4 ± 11 mmHg and 12 ± 12 mmHg and in nerve Doppler flux 17 ± 25 arbitrary units and 16 ± 34 arbitrary units, in control and diabetic rats, respectively). There was no hypotensive or tachycardic response to the subsequent ET-1 administration, although there was a sustained hypertension (maximum change in mean arterial pressure; controls 21 ± 9 mmHg and diabetics 29 ± 10 mmHg; integrated areas under the arterial pressure curve during hypertension; controls 1987 ± 812 mmHg min and diabetics 2674 ± 1280 mmHg min) and bradycardia (maximum change in heart rate; controls, -116 ± 47 beats min^{-1} and diabetics, -147 ± 52 beats min^{-1}). In response to either bosentan or ET administration, there was little difference between control and diabetic rats.

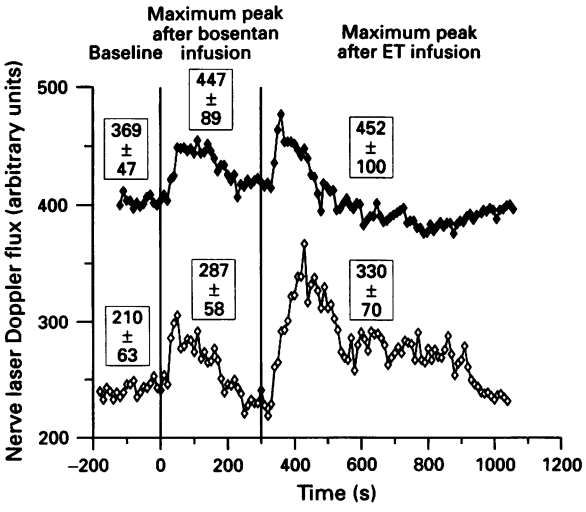

Figure 3 Typical data of nerve laser Doppler flux for one control (◆) and one diabetic (◇) rat in the acute bosentan experiment. Data in boxes are respective group means ± 1 s.d. for baseline values and maximum peaks after bosentan or endothelin (ET) administration. Bosentan administration was at 0 s and ET administration was at 300 s.

Discussion

The main aim of this study was to determine the effect of ET receptor antagonism with bosentan on sciatic nerve motor conduction velocity and laser Doppler flux -early indices of peripheral nerve dysfunction in experimental diabetes.

Untreated diabetic rats failed to gain body weight throughout the experimental protocol, resulting in reduced final weights compared to those of control animals and they were also hyperglycaemic; these are characteristics of streptozotocin-diabetes. Treatment with bosentan had no effect on these changes and thus effects on nerve function could not be attributed to alterations in the severity of diabetes in treated animals.

Untreated diabetic rats exhibited the typical slowed motor nerve conduction velocity reported many times (for example, Eliasson, 1964; Mayer & Tomlinson, 1983) and also demonstrated similar reductions in nerve laser Doppler flux to those which we have previously published (Stevens *et al.*, 1993b; 1994). The deficit of approximately 50% in nerve laser Doppler flux in diabetic rats is consistent with results from other research groups who have either used Doppler flowmetry (Yasuda *et al.*, 1989; Maxfield *et al.*, 1993; Kappelle *et al.*, 1993; Cameron *et al.*, 1994b) or other methods (Tuck *et al.*, 1984; Monafo *et al.*, 1988; Cameron *et al.*, 1991; Hotta *et al.*, 1992) to estimate nerve perfusion in experimental diabetes. The validity of Doppler flowmetry as an index of nerve blood flow requires comment and, in the aforementioned papers (Stevens *et al.*, 1993b; 1994), we discussed the limitations and fidelity of the technique. Nerve Doppler flux values are derived from the number and velocity of erythrocytes passing the probe and thus reflecting the emitted laser signal (Vongsavan & Matthews, 1993). We therefore refer to such data only as an index for whole blood flow through the nerve, but suggest that the decreases in untreated diabetic animals do reflect diminished perfusion. It has been demonstrated that peripheral nerve cannot autoregulate its blood supply (Low & Tuck, 1984) and so adequate perfusion will be dependent on systemic supply. Arterial pressures were slightly reduced in untreated diabetic rats compared to those of the control group and so, in this particular study, this may have contributed to the indicated decrease in nerve blood flow, but the proportional reduction was much greater for Doppler flux than for arterial pressure. Furthermore, daily bosentan treatment had no effect on resting systemic arterial pressure, whilst significantly increasing nerve Doppler flux, implying that the velocity of red blood cells in the sciatic nerve was increased in treated diabetic rats. This suggests an effect of chronic bosentan treatment on the vasomotor tone of the endoneurial circulation of the sciatic nerve.

The mode of action of bosentan on nerve microcirculation is debatable. As mentioned, treatment with this agent was with little effect on baseline arterial pressure and this is in agreement with work by Clozel and co-workers (1994). The apparent increase in nerve perfusion was thus presumably due to antagonism of the constrictor effect of endogenous ET in local microvessels. Such an effect could not have been sufficient to influence overall cardiovascular function, which may have been the consequence of differential antagonism of ET receptors in regional vascular beds. Kiff *et al.* (1991a, b) have shown a vasoconstriction in the hindquarters of conscious diabetic rats (in parallel with renal and mesenteric hyperaemia), which may account for the observed reductions in perfusion of peripheral nerve. Although this was not due to a hyper-responsiveness to ET (Kiff *et al.*, 1991), effective blockade of ET receptors with bosentan in this bed may have occurred with concomitant partial restoration of nerve perfusion. The effectiveness of maintained bosentan administration was demonstrated by abolition of the initial hypotensive response to a high dose of intravenous ET-1; this is in agreement with other reports, indicating blockade of ET_B receptors (Gardiner *et al.*, 1994; Clozel *et al.*, 1994). Also consistent with Gardiner *et al.* (1994) was the difficulty in detecting blockade of the marked and prolonged vasoconstriction associated with ET-1 administration. This may have been the result of several synergistic factors. With hindsight, the dose of ET-1 selected for this protocol was probably too large and, in principle, a dose-response design would have been better, but the extreme duration of the ET-1 pressor response precludes the latter approach. We therefore selected a dose based upon the extensive dose-response (over multiple animals) studies of Clozel and her colleagues (1994). A second possible reason is that the concomitant inhibition of ET-induced hypotension may have also restricted and/or masked an effect of bosentan on ET-induced vasoconstriction. Thirdly, it may be that the oral dose of bosentan was too low to antagonize completely ET_A receptors in this protocol. However, acute intravenous bosentan administration limited the extent of ET-induced hypertension, as was reported by Clozel *et al.* (1994), indicating that bosentan can antagonize ET_A receptors, provided that dose and route are optimal. It is of interest that the cardiovascular responses to ET were unchanged by diabetes, as noted by Kiff *et al.* (1991a, b). This also implies that there were no differences in the ET-provoked nitric oxide release that is thought to be responsible for the observed initial hypotension (Whittle *et al.*, 1989).

The above discussion referring to sustained bosentan treatment is supported by the effects of acute administration of this agent in animals which did not previously receive treatment. Transient increases in nerve laser Doppler flux, independent of changes in systemic arterial pressure, were evident after bosentan administration, indicating a regional effect on blood flow. Antagonism of cardiovascular responses to ET-1 administration were also seen, as discussed above. In addition, the attenuation of the raised nerve Doppler flux in response to prolonged ET-1-mediated vasoconstriction, suggests effective antagonism of ET receptors in the periphery, as noted by Gardiner *et al.* (1994).

The final consideration is the meaning of the associated prevention of reduced nerve laser Doppler flux and of motor nerve conduction velocity in the bosentan-treated diabetic rats. The high selectivity of bosentan towards ET receptors implicates ET as a causative agent in these two peripheral nerve deficits. ET is a potent vasoconstrictor, which reduces blood flow within the peripheral nerve after topical administration (Zochodne *et al.*, 1992), but has not been reported to affect the process of nerve impulse propagation. It is, therefore, likely that the two findings reported here implicate an ET-derived endoneurial vasoconstriction as the cause of the short-term motor nerve conduction deficit in diabetic rats. It is not known whether alterations in ET production, release and/or receptor numbers within the peripheral nerve in experimental diabetes contribute to this. As ET stimulates the growth of endothelial and smooth muscle cells (Kimura *et al.*, 1988), it is also possible that chronic treatment with bosentan exerted its action via effects on microvessel structure and number. To understand more fully the role of ET, our group aims to study the action of this vasoconstrictor on nerve function after direct administration to the endoneurium. The fact that bosentan did not maintain Doppler flux and conduction velocity at completely normal values in the treated diabetic rats in this study may suggest that the role of ET, although major, is not exclusive of other factors. However, there exists the possibility that a higher dose of bosentan may have prevented the deficits and, as discussed above, it may be of value to investigate a dose-response relationship. Should long-term studies indicate that the nerve conduction deficit studied here is symptomatic of the more meaningful components of diabetic neuropathy, then ET receptor blockade may have a role to play in management.

This study was supported by a grant to the William Harvey Research Institute from Ono Pharmaceuticals. We are grateful to Dr Martine Clozel for her kind gift of bosentan, to Professor Terence Bennett for constructive comments on this work and to Mark Dewhurst for guidance with establishment of the electrophysiology on the Maclab.

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(Received September 5, 1994
Revised January 26, 1995
Accepted February 10, 1995)