Originals

Angiotensin II receptor blockade improves nerve function, modulates nerve blood flow and stimulates endoneurial angiogenesis in streptozotocin-diabetic ratsand nerve function

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Summary. We examined the effect of the angiotensin II receptor blocker, ZD 8731, on nerve function, capillary density, and blood flow in streptozotocin-diabetic rats. Deficits in sciatic motor and saphenous sensory nerve conduction velocity of 21 % and 15 %, respectively, were observed after 1 month of diabetes mellitus ($p < 0.001$). These were completely ameliorated by a further month of ZD 8731 treatment ($p < 0.001$). Treatment of non-diabetic rats for 1 month with ZD 8731 had no effect on motor or sensory conduction velocity. Sciatic nerve capillary density was not significantly affected by 1- or 2-month untreated diabetes, however, there was a 15 % increase in density with ZD 8731 treatment ($p < 0.001$). Treatment of non-diabetic rats for 1 month had no effect on capillary density. Diabetes prolonged the time taken for 80 % conduction failure by 19 % ($p < 0.05$) and 49 % ($p < 0.001$) for 1 and 2 months of diabetes, respectively, when sciatic nerve was exposed to hypoxia in vitro. ZD 8731 treatment during the second month of diabetes limited the prolongation to 22 %, not significantly different from 1 month of untreated diabetes but less than for the 2-month diabetic group

($p < 0.001$). Concentrations of sciatic nerve polyol pathway metabolites were elevated six-fold and myo-inositol was reduced 40 % by diabetes; ZD 8731 treatment was without effect. Acute experiments examined the effect of ZD 8731 on sciatic nerve blood flow using laser-Doppler flowmetry. In non-diabetic rats, blood flow changes followed the dose-dependent reductions in systemic arterial pressure and there were no significant variations in sciatic vascular resistance. In marked contrast, nerve blood flow was elevated by 47 % ($p < 0.01$), and vascular resistance decreased by 32 % ($p < 0.01$) in diabetic rats despite similar changes in blood pressure compared with the non-diabetic group. Thus, the investigation has identified abnormalities in vasa nervorum reactivity which are ameliorated by angiotensin II receptor blockade and may contribute to experimental diabetic neuropathy.

Key words: Neuropathy, hypoxia, nerve blood flow, nerve conduction, capillary density, angiotensin II receptor blockade, diabetic rat.

Early changes in nerve function in rats and patients with diabetes mellitus include reduced nerve conduction velocity (NCV) and increased resistance to hypoxic conduction failure (RHCF) [1]. The vascular hypothesis for the aetiology of diabetic neuropathy attributes neurological changes to endoneurial hypoxia caused by a reduction in nutritive blood flow [1–6]. Vascular changes in diabetes are complex and may involve increased reactivity to vasoconstrictors [7] reduced endothelial nitric oxide action [8–10], the formation of advanced glycation end-products [11–13], deficiencies in essential fatty acid metabolism and prostacyclin production [14, 15], and oxidative stress [16].

Changes in the renin-angiotensin system could contribute to the vascular abnormalities of diabetes [7]. While there is some disagreement in the literature, increased plasma renin activity and elevated levels of angiotensin converting enzyme and angiotensin II have been reported

[7, 17–21]. Such alterations may be relevant to the development of early neuropathic changes because treatment with the angiotensin-converting enzyme inhibitor, lisinopril, prevented the development of motor and sensory nerve dysfunction in streptozotocin-diabetic rats [22]. The neurovascular actions of lisinopril are potentially complex; in addition to blocking angiotensin II synthesis, angiotensin converting enzyme inhibitors also potentiate bradykinin-induced vasodilation and reduce the degradation of some other vaso- or neuro-active peptides [23, 24]. Therefore, to assess whether angiotensin II-mediated vasoconstriction could contribute to neuropathy, the effects of the angiotensin II receptor antagonist, ZD 8731, on nerve function was evaluated in streptozotocin-diabetic rats.

Vasodilator treatment can cause endoneurial angiogenesis in diabetic rats, and this may be an indirect in-

Table 1. Body weights and plasma glucose concentrations for non-diabetic and diabetic rats

Group	n	Body weight (g)		Plasma glucose (mmol/l)
		Start	End	
Non-diabetic				
Control	23	483 ± 10	–	6.9 ± 0.4
ZD 8731-treated	8	455 ± 5	458 ± 6	8.6 ± 0.5
Diabetic				
1-month	10	462 ± 8	415 ± 12	36.7 ± 2.3
2-month	23	493 ± 10	371 ± 8	41.8 ± 1.9
ZD 8731-treated	13	453 ± 12	388 ± 14	40.0 ± 3.1

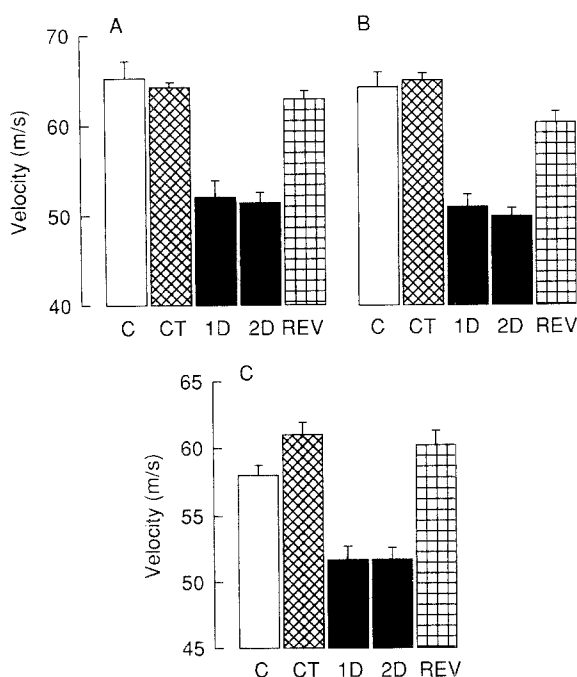Data are group means ± SEM

Fig. 1A–C. Sciatic motor nerve (**A**) gastrocnemius and (**B**) tibialis anterior conduction velocity and (**C**) saphenous sensory nerve conduction velocity in non-diabetic, untreated diabetic and diabetic rats treated with ZD 8731 after 1 month of diabetes. C, non-diabetic group, $n = 23$; CT, non-diabetic group treated for 1 month with ZD 8731 (50 mg·kg^{-1}·day^{-1}), $n = 8$; 1 D, 1-month diabetic group, $n = 10$; 2 D, 2-month diabetic group, $n = 23$; REV, ZD 8731-treated diabetic group (50 mg·kg^{-1}·day^{-1}), $n = 13$. Error bars are SEM

dicator of chronic increases in blood flow [22, 25, 26]. Thus, the effect of ZD 8731 on endoneurial capillary density was also examined. A further study, using laser-Doppler techniques, on the acute effects of ZD 8731 administration on sciatic nerve blood flow was also carried out on separate groups of diabetic and non-diabetic rats.

Materials and methods

All experiments were carried out on mature male Sprague-Dawley rats (Aberdeen University colony), 19 weeks old at the start of the study. Non-diabetic rats were used as controls, and a non-diabetic group was also treated for 1 month with ZD 8731 (Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK), a novel biphenylylmethoxy-

quinoline which is a selective AT_1 angiotensin receptor antagonist [27, 28]. ZD 8731 was dissolved in the drinking water at a concentration which resulted in rats receiving approximately 50 mg·kg^{-1}· day^{-1}. The dose was chosen to give approximately 90 % inhibition of the pressor response to a maximal angiotensin II challenge in conscious rats [27]. Other groups of rats were given streptozotocin (40–45 mg·kg^{-1} in 20 mmol·l^{-1} sodium citrate buffer, pH 4.5, i.p.). Diabetes was verified 24 h later by estimating hyperglycaemia and glucosuria (Visidex II and Diastix; Ames, Slough, UK). Samples for plasma glucose measurement were taken from the tail vein on the day of the final experiments. Diabetic animals were divided into three groups, one of which was untreated for 2 months, one was untreated for 1 month, and the third group was untreated for 1 month followed by 1 month of treatment with ZD 8731 at the dose used for non-diabetic rats.

In final experiments (1–1.5 g·kg^{-1} urethane anaesthesia i.p.), NCV was measured in vivo between the sciatic notch and the knee for the motor branches supplying the tibialis anterior (peroneal division) and gastrocnemius (tibial division) muscles. Sensory NCV was measured in the saphenous nerve between the groin and ankle. Rectal and nerve temperatures were monitored and regulated between 36.5 and 37.5 °C. The methods have previously been described in detail [29, 30].

Sciatic nerve hypoxic resistance was measured in vitro as previously described [14]. The contralateral sciatic trunk was removed and mounted on bipolar stimulating (proximal end) and recording (distal end) electrodes in a chamber containing Krebs' solution at 35 °C, with 5 mmol/l glucose for nerves from non-diabetic and 40 mmol/l glucose for nerves from diabetic rats. Bathing fluid was gassed with 95 % O_2, 5 % CO_2. Nerves were equilibrated for 30 min, then the chamber was re-filled with mineral oil pre-gassed for 1 h with 100 % N_2 and gassing was continued. Nerves were stimulated with just supramaximal pulses (1 Hz, 0.05 ms width, 10 mA) and compound action potential was monitored at 2-min intervals until it fell below 10 % of its initial value.

At the end of the experiment, the rats were killed by exsanguination. Immediately prior to this, approximately 2.5 cm of the sciatic nerve trunk, between the sciatic notch and its bifurcation at the knee, was removed and divided into five pieces which were mounted together, along with skeletal muscle which acted as a support tissue. Samples were frozen in isopentane pre-chilled in liquid nitrogen, 10-μm sections were cut on a cryostat, and capillary endothelium was stained for alkaline phosphatase using the method of Zaida et al. [25]. Three sections were taken, each 90-μm apart, and all capillaries in the nerve fascicles were counted with the aid of a projection microscope. Fascicle outlines were traced and their areas were measured using a digitizing pad linked to a micro computer.

Before exsanguination, part of the sciatic nerve proximal and distal to the sample for capillary measurements, was also taken for nerve sugar and polyol measurement and frozen in liquid nitrogen. Trimethylsilyl derivatives were prepared from aqueous deproteinized extracts and analysed by gas chromatography [31].

In a second investigation, groups of 9 non-diabetic and 13 2-month diabetic rats were used to compare the acute effects of ZD 8731 administration on systemic arterial blood pressure, sciatic nerve blood flow and vascular resistance. Rats were anaesthetized with inactin (50–150 mg·kg^{-1} i.p.). The trachea was cannulated for artificial ventilation and a carotid cannula was used to monitor mean systemic blood pressure. The jugular vein was cannulated for drug administration. The sciatic nerve was exposed between the sciatic notch and the knee. Nerve blood flow was measured using a laser-Doppler instrument (BPM^2 Laserflo, Vincent Medical, Slough, Bucks., UK) with a 0.8 mm diameter probe. This was applied under microscopic control to an area of the sciatic trunk free from large epi/perineurial vessels, care being taken not to compress the nerve. The exposed nerve was then covered with mineral oil to avoid tissue dehydration. Core temperature of the animal was monitored and regulated between 37 and 38 °C, using a rectal probe and radiant heat. Laser-Doppler flow values (arbitrary units) were allowed to reach a stable baseline over 30 min and then the effects of cumulative infusions of ZD 8731 dissolved in sterile saline (0.1–0.2 ml over

10–20 s) were monitored over 15 min periods for each dose. A concentration range of $1.5 \, ng \cdot kg^{-1}$ to $4.5 \, mg \cdot kg^{-1}$ was employed. Vascular resistance (arbitrary units) was calculated by dividing mean systemic blood pressure by blood flow using an analogue device and was continuously recorded.

Statistical analysis

Data are expressed as mean ± SEM. One-way analysis of variance was performed, followed by a Bonferroni t-test to assign differences to individual groups where overall significance ($p < 0.05$) was attained. Paired t-tests were used to assess the significance of acute within-rat changes in the second investigation.

Results

Diabetic rats exhibited hyperphagia and polydypsia. Body weights and plasma glucose concentrations are given in Table 1. Diabetic rats showed an approximately 20 % weight loss over the 2-month experimental period. Plasma glucose values were elevated approximately five-fold by diabetes. These parameters were not significantly altered by ZD 8731 treatment.

Motor NCV for sciatic branches supplying gastrocnemius (Fig. 1 A) and tibialis anterior (Fig. 1 B) was reduced by approximately 20 % after 1-month untreated diabetes ($p < 0.001$) and this was maintained over 2 months (21 % deficit; $p < 0.001$). Treatment with ZD 8731 reversed the reduction in motor NCV found with 1-month diabetes ($p < 0.01$) and the resultant values were not significantly different from those of controls. Saphenous sensory NCV (Fig. 1 C) was reduced by approximately 11 % after 1- and 2-month diabetes ($p < 0.001$), and this deficit was completely corrected by ZD 8731 treatment ($p < 0.001$ vs both diabetic groups). There were no significant effects on motor or sensory NCV for non-diabetic rats treated for 1 month with ZD 8731.

Figure 2 shows the results of sciatic endoneurial capillary density measurements. Capillary density was not significantly different for 1- or 2-month diabetic groups compared to the control group and was in the range 55.2 ± 1.5 to $58.4 \pm 1.9 \, mm^{-2}$. Treatment of diabetic rats with ZD 8731 caused a significant increase in capillary density ($p < 0.05$ vs the non-diabetic control group; $p < 0.001$ vs the 2-month diabetic group) of approximately 16 %, resulting in a value of $66.0 \pm 1.4 \, mm^{-2}$. There was no significant effect of 1-month ZD 8731 treatment on capillary density in non-diabetic rats.

Figure 3 shows the relative decline in sciatic nerve compound action potential amplitude with exposure to hypoxia in vitro. After an initial hyperexcitability phase [32] over the first 10 min of hypoxia, which was most prominent in the non-diabetic control and the 1-month diabetic groups, compound action potential amplitude declined in all groups. The decline was most rapid in nerves from non-diabetic rats, and was most prolonged in those from the 2-month diabetic group. For the 1-month and ZD 8731-treated diabetic groups, changes in compound action potential amplitude were similar, and were intermediate between control and 2-month diabetic data.
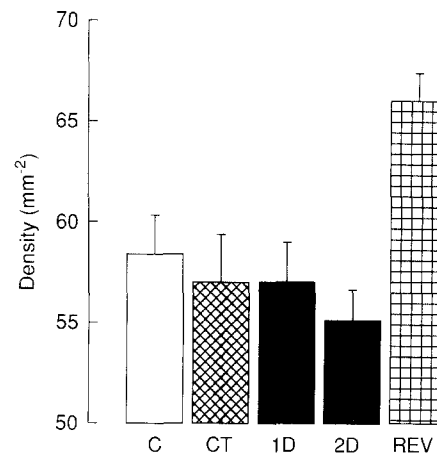

Fig. 2. Sciatic nerve endoneurial capillary density and the effects of 1-month treatment with ZD 8731 in non-diabetic and diabetic rats. C, non-diabetic group, $n = 23$; CT, non-diabetic group treated for 1 month with ZD 8731 ($50 \, mg \cdot kg^{-1} \cdot day^{-1}$), $n = 8$; 1 D, 1-month diabetic group, $n = 10$; 2 D, 2-month diabetic group, $n = 23$; REV, ZD 8731-treated diabetic group ($50 \, mg \cdot kg^{-1} \cdot day^{-1}$), $n = 13$. Error bars are SEM

The times taken for an 80 % reduction in compound action potential amplitude (T_{80}) are also shown in Figure 3. These were increased by 19 % ($p < 0.05$) and 49 % ($p < 0.001$) by 1- and 2-month diabetes respectively. ZD 8731 treatment did not reverse the effect of the initial month of untreated diabetes. Thus, T_{80} was increased by 22 % ($p < 0.01$ vs non-diabetic control group), which was not significantly different from the value for 1-month diabetic rats, but was reduced ($p < 0.001$) compared to the 2-month diabetic group.

The concentrations of sciatic nerve polyol pathway metabolites and myo-inositol are shown in Figure 4. Sorbitol (Fig. 4 A) and fructose (Fig. 4 C) were elevated six-fold by diabetes ($p < 0.001$), and myo-inositol concentration (Fig. 4 B) was decreased by 43 % ($p < 0.001$). ZD 8731 treatment did not have significant effects on sciatic nerve sorbitol, fructose or myo-inositol levels.

Figure 5 shows dose-response relationships for ZD 8731 on mean systemic arterial blood pressure, sciatic nerve blood flow and vascular resistance, measured in acute experiments on diabetic and non-diabetic rats. Both groups showed a significant dose-dependent reduction in blood pressure (Fig. 5 A) which reached 17 % and 22 % for diabetic and non-diabetic rats respectively ($p < 0.05$) for the highest dose. There were no significant differences between the two groups. Absolute levels of laser-Doppler nerve blood flow (Fig. 5 B) were reduced by 43 % in diabetic compared to non-diabetic rats ($p < 0.0001$) and vascular resistance (Fig. 5 C) showed an 80 % increase ($p < 0.002$). However, due to the limitations of the laser-Doppler technique, these values are not an accurate indicator of absolute levels of blood flow in nerve and the strength of the method lies in the measurement of relative changes [33–35]. These revealed that for diabetic rats there was a significant increase in blood flow of approximately 42 % with 4.5–45 µg/kg ZD 8731 ($p < 0.05$), although flow was reduced towards the untreated level at higher doses. In the non-diabetic group, blood flow
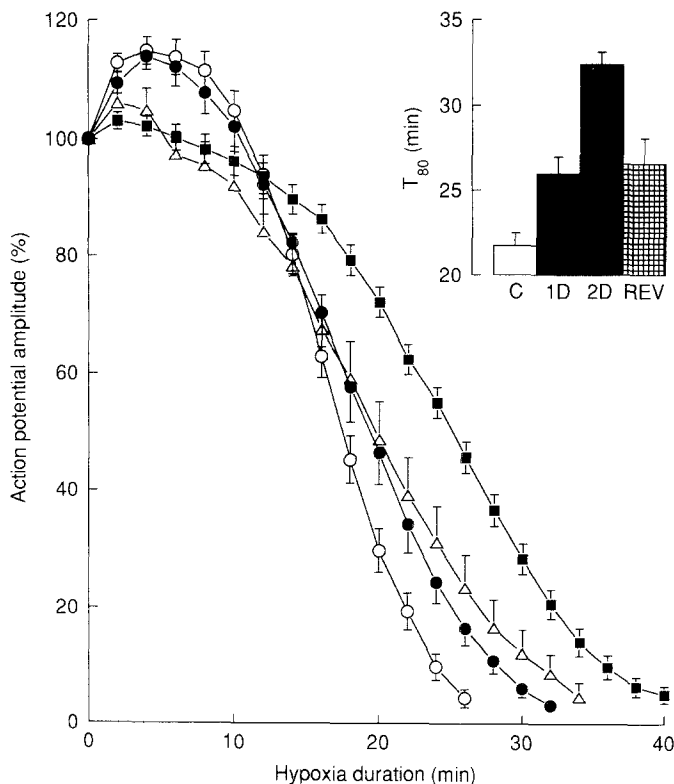
Fig. 3. Sciatic nerve resistance to hypoxia in vitro for non-diabetic and diabetic rats with and without treatment with ZD 8731 after 1 month of diabetes. Relative compound action potential amplitude is plotted against hypoxia duration. Non-diabetic group ($\bigcirc$), $n = 23$; 1-month diabetic group ($\bullet$), $n = 10$; 2-month diabetic group ($\blacksquare$), $n = 23$; ZD 8731-treated diabetic group (50 mg·kg^{-1}·day^{-1}), $n = 13$ ($\triangle$). Error bars are SEM. Statistical analysis; compared to the control group, all time-points were significantly different ($p < 0.05$) after 18 min for the 1-month and the ZD 8731-treated diabetic groups and after 14 min for the 2-month diabetic group. There were no significant differences between 1-month diabetic and ZD 8731-treated diabetic groups. One-month diabetic and ZD 8731-treated diabetic groups were significantly different ($p < 0.05$) from the 2-month diabetic group after 16 and 14 min, respectively. The inset graph shows the durations of hypoxia necessary for an 80% reduction in compound action potential (T_{80}). C, non-diabetic group, $n = 23$; 1D, 1-month diabetic group, $n = 10$; 2D, 2-month diabetic group, $n = 23$; REV, ZD 8731-treated diabetic group (50 mg·kg^{-1}·day^{-1}), $n = 13$

showed no tendency towards an increase. Instead, flow was 21% reduced ($p < 0.05$) by the highest dose of ZD 8731, and the dose-response curve approximately mirrored that for blood pressure. When variations in blood pressure were taken into account by examining sciatic vascular resistance (Fig. 5C) there were no significant differences for non-diabetic rats for any dose of ZD 8731 studied, nor any indication of dose-dependent change. In contrast, for diabetic rats, vascular resistance was reduced ($p < 0.05$) by approximately 29% for doses of ZD 8731 from 2.25 to 45 µg/kg. Although this low dose range did not result in significant reductions in systemic blood pressure in non-diabetic rats, this was not due to a lack of effectiveness of ZD 8731 as an angiotensin II receptor antagonist. Thus, in a separate set of experiments, non-diabetic rats ($n = 7$) were given graded angiotensin II challenges before and after treatment with 15 µg/kg ZD 8731 treat-

ment and the maximum elevations in systemic blood pressure were measured. The dose-response curve for angiotensin II (data not shown) was shifted to the right by treatment such that the 50% maximal effective dose was increased three-fold from 114.5 ± 17.1 to 423.4 ± 12.5 ng/kg ($p < 0.05$).

Discussion

The results demonstrate that angiotensin II receptor blockade reversed both sensory and motor NCV deficits produced by 1 month of experimental diabetes. It was without effect on NCV in non-diabetic rats. The diabetes-induced increase in RHCF was not reversed but further progression was prevented by ZD 8731 treatment. This occurred without affecting polyol pathway metabolism, which is in agreement with previous vasodilator studies [22, 36, 37]. The data also confirm the conclusion for angiotensin converting enzyme inhibitor treatment [22] that prevention of nerve dysfunction was due to inhibition of angiotensin II formation rather than potentiation of bradykinin-induced vasodilation. Furthermore, the investigation shows that inhibitors of the renin-angiotensin system have potential for treatment as well as prophylaxis.

It is plausible that vasodilators [22, 36–38] correct, or compensate for, abnormalities in vasa nervorum that lead to endoneurial hypoxia. Reports from both non-diabetic human and rat studies demonstrate that hypoxia is deleterious to peripheral nerve structure and function and that the resultant abnormalities are qualitatively similar to those observed in diabetes [39–43]. In diabetic rats, reduced sciatic blood flow and endoneurial hypoxia have been directly demonstrated [2, 4, 5, 11], and similar changes have been documented in neuropathic patients [3, 44, 45]. Furthermore, the severity of diabetic neuropathy correlates with the degree of microvascular change [46], although some microvascular abnormalities, such as basement membrane thickening, have also been found in hereditary sensory and motor neuropathy where a non-vascular aetiology is suspected [47]. Thus, it is likely that endoneurial hypoxia in diabetes causes neuropathy. Vasodilator treatment improves endoneurial blood flow in rats, with a concomitant recovery of nerve function [5]. To elucidate the aetiology of neuropathy, however, it is important to establish whether a vasodilator treatment simply compensates for unrelated vascular abnormalities, or whether it corrects one or more of them.

Plasma angiotensin II levels have been described as elevated or reduced in different studies of experimental diabetes [7, 17]. In diabetic patients, elevated angiotensin II levels [20, 21, 48] may be lowered by improved glycaemic control, however, Ferris et al. [49] measured a decrease in plasma angiotensin II in patients with neuropathy. Furthermore, increased sensitivity to angiotensin II has been noted in both diabetic rats [50] and patients [17]. Despite this, receptor density in different tissues may be reduced [48, 51, 52]. Higher circulating angiotensin converting enzyme levels are found in diabetic patients and animals [7, 53], which may reflect vascular endothelium damage. Overall, these findings indicate that diabetes
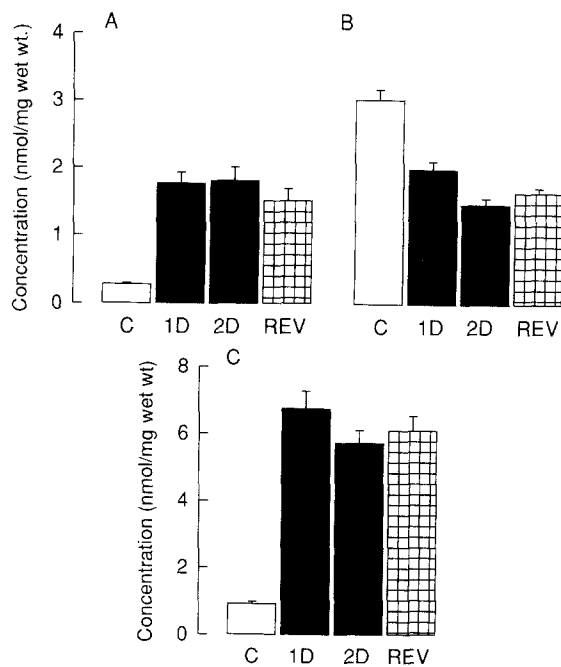
Fig. 4A–C. The effect of reversal ZD 8731 treatment on concentrations of (A) sorbitol, (B) myo-inositol and (C) fructose in sciatic nerve. C, non-diabetic group, $n = 10$; 1D, 1-month untreated diabetic group, $n = 10$; 2D, 2-month untreated diabetic group, $n = 10$; REV, ZD 8731-treated diabetic group (50 mg·kg⁻¹·day⁻¹), $n = 6$. Error bars are SEM

modifies the renin-angiotensin system, albeit in a complex and tissue-specific fashion. Other vasoactive systems may be indirectly affected by these modifications, with possible consequences for nerve blood flow. Thus, angiotensin II stimulates prostacyclin synthesis [54] and can modulate sympathetic noradrenaline release [55].

The laser-Doppler blood flow measurements revealed marked differences between diabetic and non-diabetic groups. In diabetic rats, acute ZD 8731 administration at lower doses produced an elevation in nerve blood flow and a fall in vascular resistance which was completely absent in non-diabetic rats. In contrast, both groups had similar dose-dependent reductions in blood pressure. Thus, angiotensin II blockade caused vasodilation of diabetic nerves, over and above that seen for the general circulation. Vascular resistance measurements in non-diabetic rats showed no evidence of an effect of ZD 8731 and, therefore, give no indication of a role for angiotensin II in the control of normal resting nerve blood flow. Absolute levels of vascular resistance were different in diabetic and non-diabetic rats, which could potentially complicate interpretation of the results of treatment. However, there were similar increases in blood flow in diabetic and non-diabetic rats as a result of electrical stimulation, a physiological method for producing vasodilator metabolites [4, 56]. This strongly contrasts with the findings for ZD 8731 treatment. Thus, the simplest interpretation of the data is that a diabetes-induced increase in synthesis of or sensitivity to angiotensin II contributes to elevated vasa nervorum resistance. This would reflect elevated circulating angiotensin II levels [7] in part caused by the greater activity of angiotensin converting enzyme [7, 53], however, other
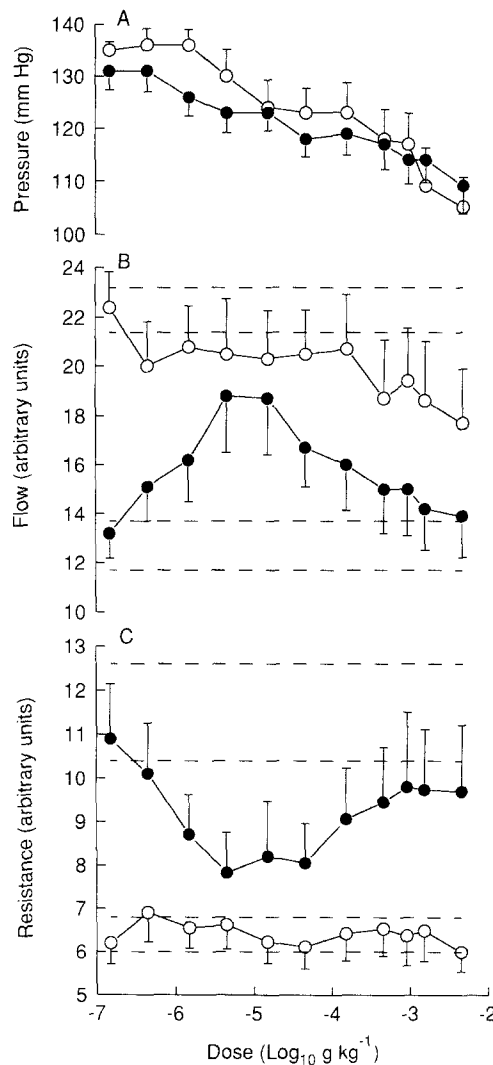

Fig. 5A–C. The effects of increasing concentrations of ZD 8731 on (A) mean systemic blood pressure, (B) sciatic blood flow and (C) sciatic vascular resistance in non-diabetic and diabetic rats. Non-diabetic group (○), $n = 9$; 2-month diabetic group (●), $n = 13$. Error bars are SEM. The horizontal dashed lines in B and C represent ± SEM around the mean for blood flow and vascular resistance in the absence of ZD 8731 administration for both groups

factors could be involved. Vascular endothelium modulates vasoconstrictor action, via the vasodilators prostacyclin and nitric oxide, mediated by specific receptors or by blood flow changes [57, 58]. In vasa nervorum there is a deficit in prostacyclin production [15] and increased sensitivity to nitric oxide inhibition suggesting reduced synthesis or action [59]. Thus, endothelium dysfunction could alter vasa nervorum reactivity to agonists such as angiotensin II in diabetes.

At high concentrations of ZD 8731, nerve blood flow and vascular resistance tended to return to resting values in diabetic rats. This corresponded to a relatively large drop in blood pressure. It seems likely at these doses that cardiovascular reflexes based on elevated sympathetic activity would produce a generalised increase in vascular resistance to partially compensate for a potentially greater drop in pressure [60], thus, a complex effect of

ZD 8731 was seen. It is of interest that this effect was not found for vasa nervorum of non-diabetic rats, although it is likely that similar generalised cardiovascular reflexes were recruited. The difference could be due to local amplification of the sympathetic response [36, 55] in diabetes, coupled with the effects of endothelium dysfunction. It is likely that such responses to acute high-dose ZD 8731 administration would not be seen for conscious diabetic rats [27] with chronic treatment as longer-term mechanisms, including increases in blood volume, would compensate for reductions in peripheral resistance in order to maintain blood pressure [60]. Thus, nerve perfusion would probably be chronically elevated in diabetic rats for the dose of ZD 8731 used in the reversal investigation, which, taking account of oral bioavailability and plasma half-life, is approximately comparable to 3 mg/kg in the acute study.

Limitations of the laser-Doppler technique for nerve blood flow measurement require comment. Absolute flow values are derived from calculations based on erythrocyte flux through the tissue, with assumptions concerning haematocrit. They reflect flow both in endoneurium and epi/perineurium, with an approximately equal contribution from each compartment [33]. There is a reasonable correlation between laser-Doppler flow values and results using other methods for within-animal comparisons, however, the correlation is relatively poor for between-animal comparisons [32–35, 61, 62]. Consequently, it is probably fortuitous, albeit suggestive, that the 43 % reduction in absolute laser-Doppler flow with diabetes agrees with previous observations of approximately 40 % decreases in nutritive endoneurial flow using hydrogen clearance [5] or whole nerve flow using ^{14}C butanol and an indicator dilution technique [4]. A further limitation is that the laser-Doppler method does not discriminate between changes in nutritive (capillary) endoneurial and non-nutritive endo-, epi- and peri-neurial flows. Given the anastomotic nature of the nerve vascular bed [63], it is possible that all flow increases with ZD 8731 administration were restricted to epi- and peri-neurium and did not affect the nutritive endoneurial component. However, the reversal study showed that ZD 8731 treatment promoted sciatic endoneurial capillary growth in diabetic rats. Chronic vasodilation causes angiogenesis in several tissues [25, 26]. Thus, it is reasonable to conclude that ZD 8731-mediated increases in endoneurial blood flow were of sufficient magnitude to both increase capillary density and improve nerve function. This is consistent with the lack of a chronic ZD 8731 treatment effect on capillarization in non-diabetic rats which paralleled the lack of acute effects on blood flow and chronic effects on nerve conduction. It must be stressed, however, that the increase in capillarization in diabetic rats is unlikely to be the cause of the reduced vasa nervorum vascular resistance. Angiogenesis is also not necessary for the amelioration of reduced NCV [16]. Thus, the major sources of vascular resistance are arterioles and feed arteries, and less powerful vasodilator treatments can restore conduction velocity without causing angiogenesis [38]. Rather, changes in capillary density give an indirect indication of chronic changes in endoneurial nutritive flow, the under-

lying mechanism being the mechanical effect on capillary endothelium [25, 26].

In conclusion, the data suggest that modifications of the renin-angiotensin system may be a factor in the aetiology of experimental diabetic neuropathy. Angiotensin II receptor blockade could have therapeutic potential, and the likely action is to improve nerve blood flow. Whether similar changes affect vasa nervorum of diabetic patients is unknown, but given the widespread use of agents which inhibit the renin-angiotensin system, it may prove worthwhile to study their potential effects on neuropathy in clinical trials.

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