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Neurovascular interactions between aldose reductase and angiotensin-converting enzyme inhibition in diabetic rats

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Abstract

Increased polyol pathway flux has been linked to nerve complications in diabetic rats, which are attenuated by aldose reductase inhibitors, defective nitric oxide-mediated vasodilation being a particular target. Diabetes also elevates the endothelial angiotensin system, increasing vasa nervorum vasoconstriction. The aim was to assess whether promotion of vasodilation by treatment with the aldose reductase inhibitor, ZD5522 (3′,5′-dimethyl-4′-nitromethylsulphonyl-2-(2-tolyl)acetanilide), coupled with reduced vasoconstriction using the angiotensin-converting enzyme inhibitor, lisinopril, interacted positively to improve neurovascular function. After 8 weeks of streptozotocin-induced diabetes, sciatic nerve blood flow and motor conduction velocity were 51% and 21% reduced, respectively. Two weeks of lisinopril treatment dose-dependently corrected the conduction deficit (ED_{50} ~0.9 mg kg^{-1}). Low-dose lisinopril (0.3 mg kg^{-1}) or ZD5522 (0.25 mg kg^{-1}) had modest corrective (10–20%) effects on nerve conduction and perfusion. However, when combined, blood flow and conduction velocity reached the nondiabetic range. The ZD5522 dose used gave a ~45% nerve sorbitol reduction but had no significant effect on fructose content; lisinopril co-treatment did not alter ZD5522 action on polyols. Thus, there was a marked neurovascular synergistic interaction between angiotensin-converting enzyme and aldose reductase inhibition in diabetic rats. This points to a potential therapeutic benefit, which requires evaluation in clinical trials.

Keywords: Neuropathy; Diabetic, rat; Aldose reductase; Angiotensin-converting enzyme; Nerve conduction; Blood flow

1. Introduction

Reduced endoneurial blood flow is a major contributory factor to the aetiology of early peripheral nerve functional abnormalities in experimental diabetes mellitus (Tuck et al., 1984; Cameron et al., 1991). For neuropathic patients, there is also compelling direct and indirect evidence for nerve perfusion defects associated with this complication (Tesfaye et al., 1994). Neurovascular abnormalities in diabetic rats can be corrected by several different drugs that either target the metabolic changes or cause vasodilatation to compensate for a dysfunctional vasa nervorum (Cameron and Cotter, 2000).

One of the notable changes in experimental diabetes is an increase in local angiotensin II release, affecting several vascular beds including vasa nervorum (Cameron et al., 1992, 1994; Maxfield et al., 1993, 1995; Cooper et al., 1994; Olbrich et al., 1996). Treatment with angiotensin-converting enzyme inhibitors and angiotensin AT_1 receptor antagonists improves nerve blood flow and conduction velocity in diabetic rats (Cameron et al., 1992, 1994a; Maxfield et al., 1993,1995; Cameron and Cotter, 1996). Clinical trials have also reported positive effects against diabetic neuropathy (Tesfaye et al., 1994; Malik et al., 1998), as well as nephropathy and retinopathy (EUCLID, 1997; Chaturvedi et al., 1998). Interestingly, in addition to reducing angiotensin II mediated vasoconstriction, chronic treatment with angiotensin-converting enzyme inhibitors appears to protect endothelial function (Olbrich et al., 1996; O'Driscoll et al., 1997). Other drugs that have metabolic actions, notably aldose reductase inhibitors (Cameron and Cotter, 1992, 1993; Tesfamariam et al., 1993) and antioxidants (Keegan et al., 1995; Rösen et al., 1995; Archibald et al., 1996) also protect vascular endothelium and improve nerve blood flow and conduction velocity (Cameron et al., 1994a,b, 1996, 1997; Hotta et al., 1995).

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Diabetes-induced endothelial dysfunction involves multiple mechanisms including elevated polyol pathway activity, increased protein kinase C activation, oxidative stress and impaired essential fatty acid metabolism. Interactions between these mechanisms are complex and synergies can be seen for some treatment combinations, for example, aldose reductase inhibitors or antioxidants combined with $n-6$ essential fatty acids (Cameron et al., 1996, 1998). The aim of this investigation was to determine whether there was an interaction between aldose reductase and angiotensin-converting enzyme inhibitor treatment by examining the effects of low doses of the aldose reductase inhibitor, ZD5522 (Cameron et al., 1994b), and lisinopril (Cameron et al., 1992), alone and in combination, on nerve blood flow and conduction velocity.

2. Materials and methods

2.1. Experimental groups and diabetes induction

Experiments were performed in accordance with regulations specified in the United Kingdom "Animal Procedures Act, 1986" and the National Institutes of Health "Principles of Laboratory Animal Care, 1985 revised version".

Male Sprague–Dawley rats were 19 weeks of age at the start of the experiments. Diabetes was induced by injection of streptozotocin (Astra-Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) at a dose of 40–45 mg kg^{-1} i.p. freshly dissolved in sterile 154 mmol l^{-1} NaCl solution. Diabetes was verified after 24 h by hyperglycaemia and glucosuria measurements (Visidex II and Diastix, Ames, Slough, UK). Plasma glucose concentrations were more accurately estimated from samples taken from the carotid cannula or by cardiac puncture on the day of experiments using a standard test kit (GOD-Perid method, Boehringer Mannheim, Mannheim, Germany).

Groups of diabetic rats were untreated for 8 weeks, or treated for the final 2 weeks after 6 weeks of untreated diabetes. In the initial part of the study, a dose–response curve for correction of sciatic motor conduction velocity was determined for lisinopril treatment (Astra-Zeneca; the drug was given in the drinking water at a dose range of 0.3 to 10 mg kg^{-1} day^{-1}). This was necessary to allow selection of an appropriate dose (causing $\sim$20% correction) for use in the interaction study. The dose–response relationship for the suphonylnitromethane aldose reductase inhibitor, ZD5122 (3′,5′-dimethyl-4′-nitromethylsulphonyl-2-(2-tolyl)acetanilide; Astra-Zeneca), had previously been established in this experimental model (Cameron et al., 1994b).

For the interaction study, using the same reversal paradigm, doses of 0.3 and 0.25 mg kg^{-1} day^{-1} were used for lisinopril and ZD5522 (daily by gavage, dissolved in distilled water), respectively. Groups were nondiabetic and diabetic controls and diabetic rats given either lisinopril, ZD5522, or combined treatments.

2.2. Sciatic nerve motor conduction velocity and endoneurial blood flow

At the end of treatment, rats were anaesthetised with thiobutabarbitone (Astra-Zeneca; 50–100 mg kg^{-1} i.p.). The trachea was cannulated for artificial ventilation and a carotid cannula was used to monitor mean systemic blood pressure. Motor conduction velocity was measured as previously described (Cameron et al., 1989) between sciatic notch and knee in the nerve branch to tibialis anterior muscle, which is representative of the whole sciatic nerve in terms of susceptibility to diabetes and treatment effects. In this model, conduction deficits are established within 2 weeks of diabetes induction, and have stabilised by 1 month without significant further alteration up to 6 months (Cameron et al., 1989, 1991).

Sciatic blood flow was measured by H_2 clearance as previously described (Cameron et al., 1996, 1998) in the nerve contralateral to the one used to estimate conduction velocity. Briefly, the nerve was exposed between sciatic notch and knee and the skin around the incision was sutured to a metal ring to form a pool that was filled with paraffin oil maintained at 35–37°C by radiant heat. Core temperature was kept within the range 37–38°C. Rats were given neuromuscular blockade using D-tubocurarine (Sigma, Poole, Dorset, UK) at a dose of 2 mg kg^{-1} via the carotid cannula, and were artificially ventilated. The level of anaesthesia was monitored by observing any reaction of blood pressure to manipulation, and supplementary anaesthetic was given as necessary. A glass-insulated platinum microelectrode was inserted into the middle portion of the sciatic nerve, above its trifurcation, and polarised at 250 mV with respect to a subcutaneous reference electrode. 10% H_2 was added to the inspired gas, the proportions of O_2 and N_2 being adjusted to 20% and 70%, respectively. When the H_2 current recorded by the electrode had stabilised, indicating equilibrium with arterial blood, the H_2 supply was shut off and N_2 delivery was increased appropriately. The H_2 clearance curve was recorded until a baseline, defined as no systematic decline in electrode current over 5 min. This procedure was then repeated at another nerve site. After the experiment, clearance curves were digitised and mono-exponential or bi-exponential curves were fitted to the data by computer using non-linear regression software that employed the Marquardt algorithm and the least squares method for optimising goodness-of-fit (Graphpad Prism, San Diego, CA, USA). The slow exponent, representing nutritive (capillary) blood flow (Day et al., 1989), was accepted. Composite flow was calculated as the weighted sum of fast and slow exponents (Cameron et al., 1996). Endoneurial nutritive vascular conductance was calculated by dividing flow by the mean arterial blood pressure during the recording period. The

averages of the two H_2 clearance determinations were taken as representative of sciatic endoneurial perfusion parameters.

2.3. Sciatic nerve polyols

Nerve samples were taken from the contralateral leg (conduction measurements) before animals were killed by exsanguination. They were frozen in liquid N_2 and stored at −80°C until subsequent analysis. Nerve sugars and polyols were determined by gas chromatography of trimethylsilyl derivatives prepared from aqueous deproteinised extracts (Stribling et al., 1985).

2.4. Statistical analysis

Data are expressed as mean ± S.E.M. They were first subjected to Bartlett's test for homogeneity of variances and were given a log transformation if necessary (vascular conductance and composite blood flow data). One-way analysis of variance was then performed, followed by Bonferroni or Student–Newman–Keuls multiple comparison tests to estimate the significance of differences for individual between-group comparisons. For the interaction study, differences between observed and predicted joint treatment effects were analysed using one sample Student *t*-tests. A value of $P < 0.05$ was considered statistically significant. The sigmoid dose–response curve for lisinopril was determined using the Marquardt algorithm and the least squares method for optimising goodness-of-fit, aided by a commercial software package (Graphpad Prism).

3. Results

Body weights (Table 1) were reduced approximately 23% and plasma glucose levels were increased by approximately sixfold in diabetic compared to the nondiabetic groups. These parameters were not significantly altered by ZD5522 or lisinopril treatments.

The dose–response curve for correction of sciatic motor conduction velocity by lisinopril is shown in Fig. 1. The degrees of amelioration of the 20.9 ± 1.0% ($P < 0.001$) diabetic deficit were 22.3 ± 4.7% ($P < 0.01$), 51.4 ± 6.7%, 82.1 ± 3.7%, and 90.0 ± 4.5% ($P < 0.001$) for 0.3, 1, 3

Table 1
Body weights and plasma glucose concentration for all rat groups used in the study
Data are mean ± S.E.M.

Group	*n*	Body weight (g)	Plasma glucose (mM)
Nondiabetic	10	446 ± 6	6.8 ± 0.6
Diabetic	10	349 ± 11	42.7 ± 1.7
Diabetic + lisinopril	31	338 ± 7	40.7 ± 1.0
Diabetic + ZD5522	12	353 ± 15	41.9 ± 1.5
Diabetic + ZD5522 + lisinopril	9	337 ± 12	39.6 ± 2.1

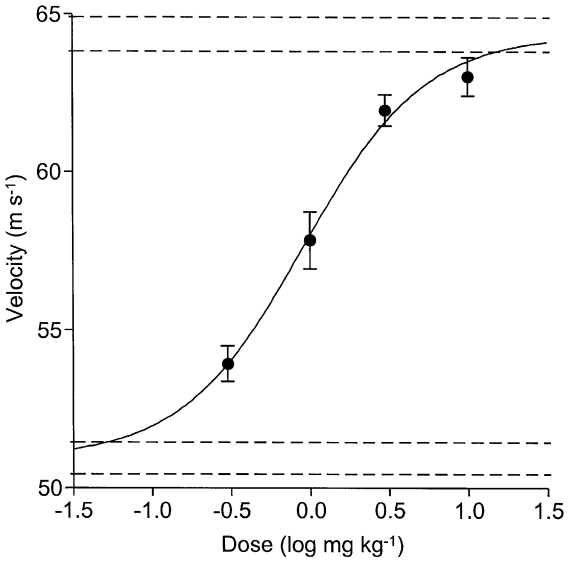

Fig. 1. Dose–response curve for correction of sciatic motor conduction velocity in diabetic rats by lisinopril treatment. Diabetes duration was 8 weeks and groups were treated with lisinopril (●) at doses of 0.3, 1.0, 3.0 and 10.0 mg kg^{-1} day^{-1} ($n = 5–6$). Data are means ± S.E.M. The dashed lines are reference values for nondiabetic (upper set) and diabetic (lower set) groups from the interaction study showing the envelope of ± S.E.M around the mean. Compared to untreated diabetes, conduction velocity was significantly ($P < 0.05$) improved by lisinopril doses of ≤ 0.3 mg kg^{-1} day^{-1} and was not significantly different from the nondiabetic control range for doses of 3 and 10 mg kg^{-1} day^{-1}.

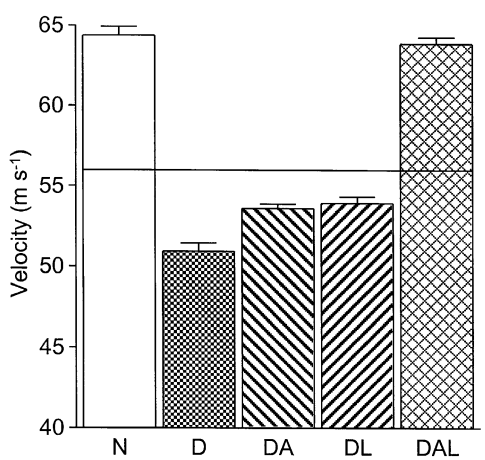

Fig. 2. Effects of diabetes and treatments containing low doses of ZD5522 and lisinopril, alone and in combination, on sciatic motor conduction velocity. N, nondiabetic group, $n = 10$; D, 8-week diabetic control group, $n = 10$. Other groups were diabetic for 8 weeks and treated for the last 2 weeks with either 0.25 mg kg^{-1} day^{-1} ZD5522 (DA, $n = 12$), 0.3 mg kg^{-1} day^{-1} lisinopril (DL, $n = 10$), or combined ZD5522/lisinopril treatment (DAL, $n = 9$). Data are mean ± S.E.M. The horizontal line shows the expected conduction velocity level for combined treatment in the DAL group, calculated from DA and DL group data assuming additivity of effects. Statistical analysis: N vs. D, DA or DL, $P < 0.001$; D vs. DA, DL, or DAL, $P < 0.001$; DAL vs. DA or DL, $P < 0.001$; all other comparisons NS.

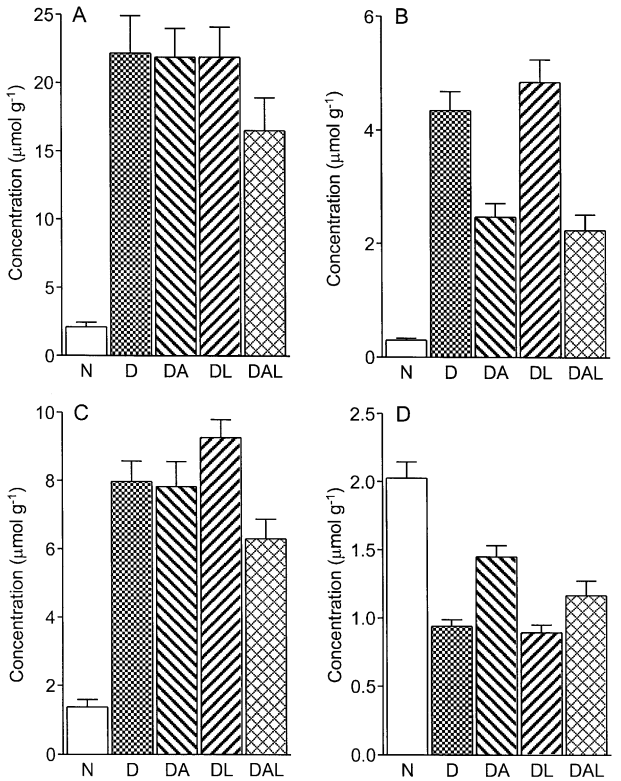

Fig. 3. (A–D) Effects of diabetes and treatments containing low doses of ZD5522 and lisinopril, alone and in combination, on sciatic nerve carbohydrates: (A) glucose, (B) sorbitol, (C) fructose, and (D) myo-inositol. N, nondiabetic group, $n = 10$; D, 8-week diabetic control group, $n = 10$. Other groups were diabetic for 8 weeks and treated for the last 2 weeks with either 0.25 mg kg^{-1} day^{-1} ZD5522 (DA, $n = 12$), 0.3 mg kg^{-1} day^{-1} lisinopril (DL, $n = 8$), or combined ZD5522/lisinopril treatment (DAL, $n = 9$). Data are mean ± S.E.M.

and 10 mg kg^{-1}, respectively. For the 10 mg kg^{-1} dose, conduction velocity was not significantly different from the nondiabetic range. The log ED_{50} was -0.039 ± 0.037 log mg kg^{-1}, which corresponds to a dose of approximately 0.9 mg kg^{-1}. A lisinopril dose of 0.3 mg kg^{-1} was chosen for the interaction study with ZD5522.

Conduction data for the interaction study are shown in Fig. 2. Low-dose ZD5522 (0.25 mg kg^{-1}) or lisinopril (0.3 mg kg^{-1}) treatments alone corrected the diabetic deficit by $19.9 \pm 2.0\%$ and $22.4 \pm 2.9\%$ ($P < 0.001$), respectively. When treatments were combined, the degree of correction was $96.1 \pm 3.1\%$ ($P < 0.001$) and the resultant conduction velocity value of 63.9 ± 0.4 m s^{-1} greatly exceeded ($P < 0.0001$) the prediction of 56.0 m s^{-1} for addition of the effects of ZD5522 and lisinopril from the single treatment group data. This conduction effect was equivalent to that predicted for a dose of ~ 16 mg kg^{-1} from the lisinopril dose–response curve (Fig. 1).

Analysis of sciatic nerve carbohydrates (Fig. 3) showed that glucose was elevated approximately 9.8-fold by diabetes ($P < 0.001$) and this was not significantly altered by treatment. Sorbitol levels were increased 14.8-fold by diabetes ($P < 0.001$); this was not affected by lisinopril treatment, however, ZD5522 reduced sorbitol ($P < 0.001$) by $43.2 \pm 5.5\%$ and $48.5 \pm 6.3\%$ in the single and joint treatment groups, respectively. Fructose was 5.8-fold elevated by diabetes and this was not significantly affected by ZD5522 or lisinopril treatments, alone or in combination. Nerve myo-inositol content was $53.7 \pm 2.5\%$ reduced by diabetes ($P < 0.001$) and this was unaffected by lisinopril. ZD5522 partially corrected ($46.9 \pm 7.7\%$; $P < 0.001$) the myo-inositol deficit in the single treatment group although a trend towards correction in the joint treatment group was not statistically significant.

Nutritive (capillary) endoneurial blood flow (Fig. 4A) was $50.7 \pm 3.1\%$ ($P < 0.001$) reduced by diabetes. There were trends in the direction of correction to the extent of

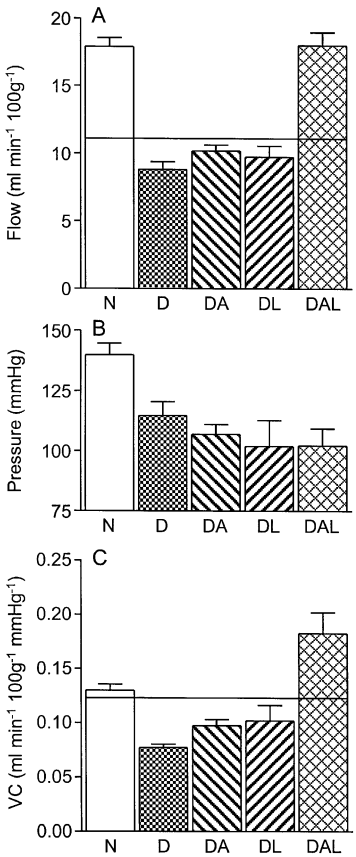

Fig. 4. (A–C) Effects of diabetes and treatments containing low doses of ZD5522 and lisinopril, alone and in combination, on (A) sciatic endoneurial nutritive blood flow, (B) mean systemic blood pressure and (C) endoneurial nutritive vascular conductance (VC). N, nondiabetic group, $n = 10$; D, 8-week diabetic control group, $n = 10$. Other groups were diabetic for 8 weeks and treated for the last 2 weeks with either 0.25 mg kg^{-1} day^{-1} ZD5522 (DA, $n = 12$), 0.3 mg kg^{-1} day^{-1} lisinopril (DL, $n = 8$), or combined ZD5522/lisinopril treatment (DAL, $n = 9$). Data are mean ± S.E.M. The horizontal lines in A and C show the expected levels for combined treatment in the DAL group, calculated from DA and DL group data assuming additivity of effects. Statistical analysis: flow; N vs. D, DA or DL, $P < 0.001$; DAL vs. D, DA or DL, $P < 0.001$. Pressure; N vs. D, DA. DL or DAL, $P < 0.01$. Vascular conductance; N vs. D, $P < 0.001$; N vs. DA or DAL, $P < 0.01$; N vs. DL, $P < 0.05$; DAL vs. D, DA or DL, $P < 0.001$. All other comparisons NS.

15.0 ± 5.1% and 10.0 ± 9.0% for ZD5522 and lisinopril treatments alone, although these were not statistically significant. In contrast, blood flow was within the nondiabetic range for joint treatment ($P < 0.001$ vs. diabetic control and single treatment groups). The resultant flow value of 18.0 ± 1.0 ml min^{-1} 100 g^{-1} markedly exceeded ($P = 0.0001$) the prediction of 11.1 ml min^{-1} 100 g^{-1} derived from addition of the incremental flow increases from the single treatment groups. Mean systemic blood pressure (Fig. 4B) was reduced by 18.0 ± 4.2% to 27.2 ± 7.7% ($P < 0.01$) in diabetic control and treated diabetic groups, which would alter blood flow as vasa nervorum has poor pressure autoregulation (Day et al., 1989). To take account of this, perfusion data are also expressed as vascular conductance (Fig. 4C). There was a 40.6 ± 2.4% ($P < 0.001$) reduction in vascular conductance with diabetes and there were numerical trends towards correction for ZD5522 and lisinopril treatments (38.5 ± 10.8% and 46.9 ± 27.1%, respectively) which were not statistically significant. In the joint treatment group, however, vascular conductance was supernormal, exceeding that of the nondiabetic control group by 40.8 ± 14.9% ($P < 0.01$). The value of 0.183 ± 0.019 ml min^{-1} 100 g^{-1} mm Hg^{-1} also exceeded ($P = 0.012$) the additive predicted value of 0.122 ml min^{-1} 100 g^{-1} mm Hg^{-1} derived from the single treatment data.

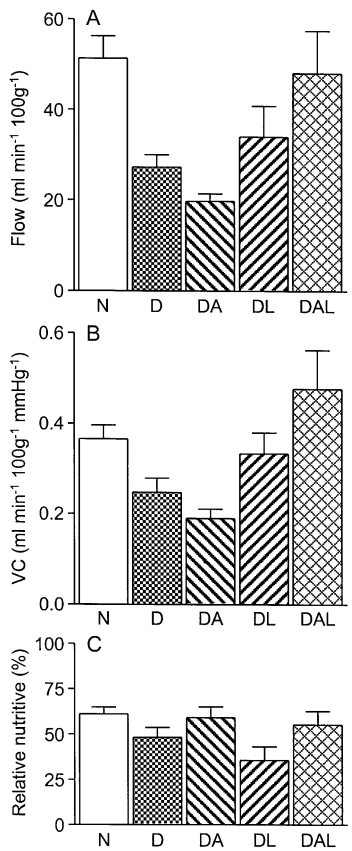

Fig. 5. (A–C) Effects of diabetes and treatments containing low doses of ZD5522 and lisinopril, alone and in combination, on (A) sciatic composite endoneurial blood flow, (B) composite vascular conductance and (C) the percentage of nutritive flow. N, nondiabetic group, $n = 10$; D, 8-week diabetic control group, $n = 10$. Other groups were diabetic for 8 weeks and treated for the last 2 weeks with either 0.25 mg kg^{-1} day^{-1} ZD5522 (DA, $n = 12$), 0.3 mg kg^{-1} day^{-1} lisinopril (DL, $n = 8$), or combined ZD5522/lisinopril treatment (DAL, $n = 9$). Data are mean ± S.E.M. Statistical analysis: flow; N vs. DA, $P < 0.001$; N vs. D, $P < 0.01$; N vs. DL, $P < 0.05$; DAL vs. D, $P < 0.05$; DAL vs. DA, $P < 0.001$; DA vs. DL, $P < 0.05$. Vascular conductance: N vs. DA, $P < 0.01$; DAL vs. D, $P < 0.01$; DAL vs. DA, $P < 0.001$; DA vs. DL, $P < 0.01$. Percentage nutritive flow: DL vs. N or DA, $P < 0.05$. All other comparisons NS.

Hydrogen clearance curves for peripheral nerve are usually composed of two simultaneously recorded components. A fast component arises due to clearance by large vessels (non-nutritive arterial, venous and particularly arteriovenous flow), and a slow component appears as the result of nutritive (capillary) clearance (Day et al., 1989). Data for composite (total endoneurial) flow and conductance are given in Fig. 5A and B, respectively. Composite flow was 46.9 ± 5.4% ($P < 0.01$) reduced by diabetes and this was not significantly altered in the ZD5522 or lisinopril single treatment groups. However, for joint treatment, flow was in the nondiabetic range, significantly increased compared to diabetic control ($P < 0.01$) and ZD5522-treated diabetic ($P < 0.001$) groups, but not the lisinopril-treated group. Similar treatment effects were apparent for vascular conductance. The percentage of H_2 clearance via nutritive perfusion (Fig. 5C), an index of the pattern of endoneurial blood flow and degree of arteriovenous shunting (Day et al., 1989; Cameron et al., 1994b) was not significantly affected by diabetes, ZD5522 or joint treatment. However, in the lisinopril treatment group, the percentage nutritive clearance was ~40% lower ($P < 0.05$) than for nondiabetic control and ZD5522-treated diabetic groups.

4. Discussion

Lisinopril treatment caused dose-dependent nerve conduction velocity increases in diabetic rats, in agreement with previous investigations using angiotensin-converting enzyme inhibitors (Cameron et al., 1992; Kihara et al., 1999). This effect may be mimicked by angiotensin AT_1 receptor antagonists (Maxfield et al., 1993, 1995; Cameron and Cotter, 1996), suggesting that angiotensin-converting enzyme inhibitor action depends upon blocking angiotensin II formation, rather than potentiating bradykinin-mediated responses because of reduced degradation. The efficacy of high-dose lisinopril in completely correcting conduction deficits supports a vascular aetiology for experimental diabetic neuropathy.

Increased endothelial angiotensin II synthesis and increased vascular and plasma angiotensin-converting enzyme levels may be in part caused by elevated oxidative stress in diabetes, being reduced by antioxidants (Cameron et al., 1994a; Cooper et al., 1994). Vasa nervorum also

shows increased angiotensin II mediated vasoconstriction (Maxfield et al., 1993); at least three factors may contribute. First, the nitric oxide (NO) system is impaired by diabetes; therefore, a braking vasodilator mechanism to limit vasa nervorum vasoconstriction is dysfunctional (Maxfield et al., 1997; Kihara et al., 1999). Physiological levels of NO also inhibit angiotensin-converting enzyme, which would normally limit angiotensin II formation (Ackermann et al., 1998). Second, vascular protein kinase C activity is elevated by diabetes and protein kinase C is a mediator of angiotensin II-induced vascular smooth muscle contraction (Oriji and Keiser, 1997; Cameron et al., 1999). Third, endothelin-1 production is increased by diabetes, and this acts synergistically to enhance angiotensin II mediated vasoconstriction in vasa nervorum (Cameron and Cotter, 1996).

The vascular endothelium and NO vasodilator systems are major aldose reductase inhibitor targets in diabetes, as has been shown in several vessels and vascular beds including vasa nervorum (Cameron and Cotter, 1992; Tesfamariam et al., 1993; Cameron et al., 1996; Keegan et al., 2000). Prevention or correction of NO deficits could depend upon increased availability of NADPH, which is a co-factor common to aldose reductase and NO synthase (Cameron and Cotter, 1992, 1993). However, it is not clear whether this putative mechanism is rate limiting for NO synthase in endothelial cells. A plausible alternative explanation is based on indirect action of aldose reductase inhibitors as antioxidants, because they correct a diabetic depression in tissue glutathione synthesis (Cameron and Cotter, 1999; Obrosova et al., 1999). In turn this would improve free radicals scavenging, to limit destruction of NO by reactive oxygen species. Such an effect is seen with scavenger or transition metal chelator treatments in several vessels and vascular beds in diabetic rats, including vasa nervorum (Cameron et al., 1994a; Keegan et al., 1995, 1999a,b; Rösen et al., 1995). There are multiple sources of oxidative stress in diabetes, including sugar autoxidation, the advanced glycosylation process, mitochondrial abnormalities, ischaemia–reperfusion effects and the vascular NAD(P)H oxidase system (Cameron and Cotter, 1999). The latter may be particularly pertinent to this investigation, because angiotensin II stimulates constitutive NADPH oxidase in vessels, so elevating superoxide production (Pagano et al., 1997). This could neutralise NO, forming peroxynitrite and favouring release of highly reactive hydroxyl radical that could cause endothelial damage (Beckman et al., 1990).

Aldose reductase inhibitors and antioxidants also appear to have beneficial effects on endothelial vasodilator function that extend beyond the NO system. Thus, aldose reductase inhibitor and lipoic acid treatments partially protected against the development of a gross deficit in endothelium derived hyperpolarising factor, and aldose reductase inhibitors and vitamin E improved endothelial prostacyclin synthesis (Karpen et al., 1982; Wakasugi et al., 1991; Cameron and Cotter, 1999; Keegan et al., 2000). This profile of effects is likely to be responsible for the improvements in nerve perfusion seen with aldose reductase inhibitor treatment, which has functional consequences for conduction velocity (Cameron et al., 1994b, 1996, 1997; Hotta et al., 1995). In contrast, interest in the endothelial effects of renin–angiotensin system antagonists in diabetes has focussed on the beneficial effects on the NO mechanism (Olbrich et al., 1996; O'Driscoll et al., 1997; Kihara et al., 1999). In conjunction with anti-vasoconstrictor actions on smooth muscle, this could be responsible for the effects on neurovascular function in diabetic rats (Cameron et al., 1992; Maxfield et al., 1993, 1995).

It is plausible that the synergistic effect of joint lisinopril ZD5522 treatment on sciatic nerve nutritive blood flow and conduction velocity could depend on a "push–pull" effect involving reduced angiotensin II-mediated vasoconstriction coupled with improved vasodilation by multiple endothelial mediators. Singly, these two agents did not have identical effects on vasa nervorum as suggested by the nerve perfusion measurements. Thus, for ZD5522 there were nonsignificant trends toward increased nutritive flow perhaps at the expense of composite flow, which is in agreement with the statistically clear picture seen with high-dose aldose reductase inhibitor treatment (Cameron et al., 1994b, 1996, 1997). However, lisinopril tended to increase both nutritive and composite flow, biased towards the latter. For joint treatment, these opposing effects tended to cancel, the resulting flow distribution being essentially normal.

Another potential theoretical basis for synergistic interactions on nerve function depends on direct aldose reductase inhibitor actions on nerve fibres/Schwann cells themselves. This manifests as effects on polyol content, which mirrors actions on nerve maximum ouabain-sensitive Na^+, K^+-ATPase activity (Basso et al., 1998). The low-dose ZD5522 treatment used in this study halved nerve sorbitol levels but had only a minor effect on conduction velocity, in agreement with previous investigations of aldose reductase inhibitor dose–response relationships (Cameron et al., 1994b). The decrease in nerve myo-inositol with diabetes was also partially restored by aldose reductase inhibitor treatment, in line with the complementary osmolyte hypothesis (Stevens et al., 1993). Improvements in myo-inositol to this degree lead to elevated Na^+, K^+-ATPase activity, although this measure correlates only poorly with changes in conduction velocity (Cameron et al., 1999). It is possible that any Na^+, K^+-ATPase activity increase with aldose reductase inhibition was, on its own, insufficient to improve conduction velocity in the face of a sustained deficit in endoneurial blood flow. However, further correction of the blood flow deficit by lisinopril co-treatment, enhanced by the synergistic vascular interaction, would result in improved oxygen supply to nerve such that sufficient ATP was available for Na^+, K^+-ATPase, and nerve conduction was improved.

Thus, the synergistic interaction between aldose reductase and angiotensin-converting enzyme inhibition on nerve function could potentially be explained by purely vascular or mixed vascular/neuronal actions. The consequence is to markedly amplify drug efficacy. If these mechanisms are applicable to patients with diabetes, they should provide a therapeutic advantage, which could be examined in clinical neuropathy trials. The patients most likely to benefit from this approach would be those with mild or subclinical neuropathy and little evidence of overt angiopathy. Thus, the nerves of patients with clinical neuropathy show much more advanced degenerative changes than those in experimental diabetes and the degree to which this may be reversed by treatment is not known (Tesfaye et al., 1994; Cameron and Cotter, 2000). Furthermore, the presence of extensive endothelial damage and atherosclerosis could limit the potential advantages of a combined aldose reductase, angiotensin-converting enzyme inhibitor approach.

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