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Correction of neurovascular deficits in diabetic rats by β_2-adrenoceptor agonist and α_1-adrenoceptor antagonist treatment: Interactions with the nitric oxide system

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Abstract

The aims were to test whether 2 weeks treatment with the β_2-adrenoceptor agonist, salbutamol, or the α_1-adrenoceptor antagonist, doxazosin, could correct nerve blood flow and conduction velocity deficits in 8 week streptozotocin-diabetic rats and to examine neurovascular mechanisms using co-treatment with the nitric oxide synthase inhibitor, N^G-nitro-L-arginine. Sciatic motor conduction velocity, 20.3% reduced by diabetes, was corrected by 88.2 and 88.5% for salbutamol and doxazosin, respectively. A 47.6% diabetic deficit in sciatic nutritive endoneurial blood, was substantially reversed by salbutamol (117.0%) and doxazosin (61.0%) treatment. The effects of α_1-adrenoceptor blockade and β_2-adrenoceptor stimulation on nerve blood flow and conduction velocity were almost completely (76.7–91.7%) attenuated by N^G-nitro-L-arginine co-treatment. Thus, the data stress the importance of vasa nervorum α_1 and β_2 adrenoceptors and the permissive role of nitric oxide in nerve blood flow control mechanisms. They also indicate that β_2-adrenoceptor agonists may be suitable for clinical trials of diabetic neuropathy. © 1998 Elsevier Science B.V.

Keywords: Nerve conduction; Blood flow; α_1-Adrenoceptor; β_2-Adrenoceptor; Nitric oxide (NO); Neuropathy; Diabetic rat

1. Introduction

Reduced peripheral nerve conduction velocity in experimental diabetes is linked to decreased blood flow and endoneurial hypoxia (Tuck et al., 1984; Low et al., 1989; Cameron et al., 1991b). In neuropathic diabetic patients nerve perfusion is reduced, the endoneurium is hypoxic, and the condition is aggravated by overt peripheral vascular disease (Ram et al., 1991; Tesfaye et al., 1994). One therapeutic approach is to improve nerve blood flow with peripheral vasodilator treatment, which was successful in diabetic rats (reviewed in Cameron and Cotter, 1994) and produced some benefits for nerve function in neuropathic patients (Reja et al., 1995). In rat models, effective vasodilators include those targeting the sympathetic noradrenergic system (Cameron et al., 1991a,b; Cotter and Cameron, 1995; Dines et al., 1995a) which is important for the neural control of blood flow via α-adrenoceptors on the epineurial vessels of vasa nervorum (Kihara and Low, 1990). Although α_1-adrenoceptor antagonists such as prazosin improve conduction velocity in diabetic rats (Cameron et al., 1991b), their effect on the nerve blood flow deficit has not previously been examined. In addition, while β-adrenoceptor-mediated vasodilation is found in a number of vascular beds including skeletal muscle and brain (Belfrage, 1978; Lass et al., 1989), it is not known whether this system is sufficiently present in vasa nervorum to affect endoneurial blood flow. Therefore, one aim was to compare the actions of the β_2-adrenoceptor agonist, salbutamol, and the α_1-adrenoceptor antagonist, doxazosin, on nerve perfusion and conduction deficits in diabetic rats.

Nerve blood flow and function defects in diabetic rats are also corrected by inhibitors of some metabolic changes (reviewed in Cameron and Cotter, 1994), including increased oxygen free radical production, the formation of advanced glycation end products, elevated aldose reductase activity and altered essential fatty acid and L-carnitine metabolism (Kihara et al., 1991; Bravenboer et al., 1992; Dines et al., 1995b; Cameron and Cotter, 1997; Cameron et al., 1997). In turn, some of the metabolic changes have

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been linked to reduce nitric oxide production or action by vascular endothelium (Cameron and Cotter, 1992; Keegan et al., 1995; Rösen et al., 1995; Archibald et al., 1996) and the beneficial neurovascular effects of drugs targeting these metabolic mechanisms are attenuated by co-treatment with nitric oxide synthase inhibitors (Stevens et al., 1994; Cameron and Cotter, 1995, 1996a,b; Cameron et al., 1996). However, some drugs, for example myo-inositol, might act 'downstream' of the endothelium and may still correct nerve conduction velocity deficits in the presence of nitric oxide synthase inhibition (Stevens et al., 1994). α_1-Adrenoceptor antagonists act downstream of the endothelium, directly on vascular smooth muscle. Thus, on a simple view, one prediction is that their action could persist in the presence of nitric oxide synthase inhibition, particularly as the vasa nervorum nitric oxide system is already impaired by diabetes (Kihara and Low, 1995; Maxfield et al., 1997). Vasodilation by β_2-adrenoceptor agonists, if present in vasa nervorum, may be endothelium-independent or partially endothelium-dependent by stimulating the nitric oxide system (Kamata et al., 1989; Gardiner et al., 1991; Wang et al., 1993; Randall and McCulloch, 1995; Rebich et al., 1995). Co-treatment with a nitric oxide synthase inhibitor could in principle be used to distinguish between these two modes of action. Alternatively, vasodilator responses could be augmented by flow-induced stimulation of vasa nervorum endothelial nitric oxide production (Fujii et al., 1992), in which case nitric oxide synthase inhibitor co-treatment would attenuate the effects of both α_1-adrenoceptor antagonists and β_2-adrenoceptor agonists. Thus, to examine potential interactions with the nitric oxide system, we studied the effects of co-treatment with the nitric oxide synthase inhibitor, N^G-nitro-L-arginine, in response to salbutamol and doxazosin.

2. Materials and methods

Male Sprague–Dawley rats (Aberdeen University breeding colony), 19 weeks old at the start of the study were used. Nondiabetic animals acted as onset controls. Diabetes was induced by intraperitoneal administration of streptozotocin (Zeneca, Macclesfield, Cheshire, UK) at 40–45 mg kg^{-1}, freshly dissolved in sterile saline. Diabetes was verified 24 h later by estimating hyperglycaemia and glycosuria (Visidex II and Diastix; Ames, Slough, UK). Diabetic rats were tested weekly and weighed daily. Animals were rejected if the plasma glucose concentration was < 20 mM or if body weight consistently increased over 3 days. Samples were taken from the carotid cannula on the day of final experiments in order to measure plasma glucose concentration (GOD-Perid method; Boehringer Mannheim, Mannheim, Germany).

After 6 weeks of untreated diabetes, groups of rats were untreated or treated for 2 weeks with doxazosin (Pfizer, Sandwich, Kent, UK) or salbutamol (Sigma, Poole, Dorset, UK) without or with co-treatment with N^G-nitro-L-arginine (Sigma), dissolved in the drinking water such that doses were approximately 10, 0.3 and 10 mg kg^{-1} day^{-1}, respectively. A group of nondiabetic rats was also treated with salbutamol for 2 weeks. The doses were chosen based on previous studies using prazosin and doxazosin in diabetes-related models of nerve dysfunction (Cameron et al., 1991b; Dines et al., 1995a), N^G-nitro-L-arginine in nondiabetic and diabetic rats (Cameron et al., 1993, 1996) and pilot studies for salbutamol (Cotter and Cameron, unpublished observations). At the dose of sabutamol used, there were no significant effects on heart rate in diabetic (259 ± 13 min^{-1} untreated, 248 ± 5 min^{-1} treated) and nondiabetic (347 ± 11 min^{-1} untreated, 351 ± 12 min^{-1} treated) rats, monitored during blood flow measurements, indicating minimal β_1-adrenoceptor-mediated stimulation.

At the end of the treatment period, rats were anaesthetised with thiobutabarbital (Zeneca) by intraperitoneal injection (50–100 mg kg^{-1}). 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. Rectal and nerve temperatures were regulated between 36.5 and 37.5°C.

Sciatic endoneurial blood flow was then measured in the contralateral leg using microelectrode polarography and hydrogen clearance as previously described (Cameron et al., 1991b, 1996). Briefly, rats were given neuromuscular blockade using D-tubocurarine (Sigma, 2 mg · kg^{-1} via the carotid cannula) and artificially ventilated. The level of anaesthesia was monitored by observing any reaction of blood pressure to manipulation, and supplementary thiobutabarbitone was given as necessary. A glass-insulated H_2-sensitive platinum microelectrode was inserted into the middle portion of the sciatic nerve. 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 electrode H_2 current had stabilised, the H_2 supply was shut off and the clearance curve monitored. This was repeated at another nerve site > 4 mm proximal or distal to the original site. Sciatic nerve temperature was kept in the range 35–37°C by radiant heat applied to a mineral oil pool that bathed the exposed nerve. Mono- or bi-exponential curves were fitted to the data by regression (Prism, Graphpad, San Diego, CA). The slow exponent was taken to reflect nutritive capillary flow and the fast exponent non-nutritive flow (Low et al., 1989). Composite (total) endoneurial flow was defined as the weighted sum of fast and slow components. The percentage of nutritive clearance was determined from the weighting coefficients for slow and fast components. Vascular conductance was calculated by dividing blood flow by mean arterial blood pressure during the recording period.

2.1. Statistical analysis

Data are expressed as group means ± S.E.M. They were subjected to Bartlett's test for homogeneity of variance, followed by log transformation if appropriate (nutritive vascular conductance, composite blood flow and conductance), and then one-way analysis of variance. If a significant ($P < 0.05$) effect was found, between group differences, corrected for multiple comparisons, were identified using the Student–Newman–Keuls test.

3. Results

Diabetic rats had an approximately 5-fold elevation of non-fasted plasma glucose and lost approximately 29% of their body weight over 8 weeks (Table 1). These parameters were not significantly affected by treatment with doxazosin, salbutamol or N^G-nitro-L-arginine during the final 2 week period.

Sciatic motor conduction velocity to tibialis anterior muscle (Fig. 1) was 20.3 ± 1.0% reduced by 8 weeks of diabetes ($P < 0.001$). Doxazosin and salbutamol treatment during the last 2 weeks corrected the conduction deficit by 88.5 ± 4.3 and 88.2 ± 4.0%, respectively (both $P < 0.001$). The resulting values did not differ significantly from those of the nondiabetic group. Salbutamol did not alter conduction velocity in nondiabetic rats. When N^G-nitro-L-arginine was given jointly with these drugs, their effect on conduction velocity was markedly blunted. Thus the improvements with doxazosin and salbutamol were reduced to 20.6 ± 4.3 and 12.7 ± 7.7%, respectively ($P < 0.001$), the former but not the latter remaining statistically significant ($P < 0.05$) compared to the diabetic control group.

Endoneurial nutritive blood flow (Fig. 2A) was 47.6 ± 2.3% decreased by diabetes ($P < 0.001$). Doxazosin treatment corrected the flow deficit by 61.0 ± 8.5% ($P < 0.001$). Salbutamol treatment elevated flow in diabetic rats ($P < 0.001$) to a value that was 17.0 ± 4.8% ($P < 0.05$) greater than in the nondiabetic control group. For salbutamol treated nondiabetic rats, endoneurial nutritive flow also exceeded the untreated control group value by a similar amount (15.7 ± 4.9%, $P < 0.01$). In salbutamol and doxazosin treated diabetic groups, co-treatment with

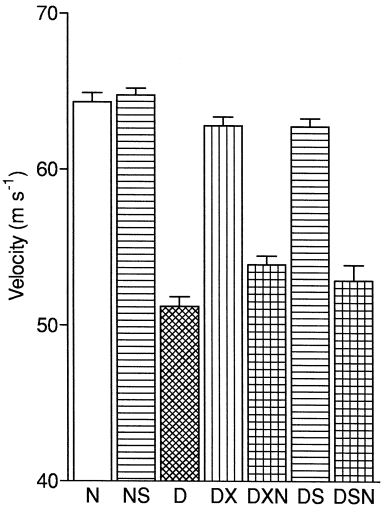

Fig. 1. Sciatic nerve motor conduction velocity in fibres supplying tibialis anterior muscle. N, nondiabetic control group; NS, nondiabetic group treated with salbutamol (0.3 mg kg^{-1} day^{-1}) for 2 weeks; D, 8-week diabetic control group; DX, 8-week diabetic group treated for the last 2 weeks with doxazosin (10 mg kg^{-1} day^{-1}); DXN, 8-week diabetic group treated for the last 2 weeks with both doxazosin and N^G-nitro-L-arginine (10 mg kg^{-1} day^{-1}); DS, 8-week diabetic group treated for the last 2 weeks with salbutamol; DSN, 8-week diabetic group treated for the last 2 weeks with both salbutamol and N^G-nitro-L-arginine. Group $n = 8–10$. Error bars are S.E.M.

N^G-nitro-L-arginine reduced nutritive flow ($P < 0.001$) to values within the untreated diabetic range.

Mean systemic blood pressure (Fig. 2B) was reduced by 16.8 ± 2.7% with untreated diabetes ($P < 0.01$) and this was unaffected by salbutamol treatment. Doxazosin caused a further (19.1 ± 3.8%, $P < 0.01$) hypotensive effect compared to the untreated diabetic group, which was restored by N^G-nitro-L-arginine co-treatment ($P < 0.01$). Blood pressure was not further increased by N^G-nitro-L-arginine in salbutamol treated diabetic rats. Salbutamol did not significantly alter blood pressure in nondiabetic rats. Vasa nervorum shows minimal pressure autoregulation (Low et al., 1989), therefore, flow values determined during the experiments would have been influenced by the between-group variations in blood pressure. To take account of this, the perfusion data are also expressed as endoneurial vascu-

Table 1
Body weights and plasma glucose concentrations

Group	*n*	Body weight		Plasma glucose (mM)
		start (g)	end (g)	
Nondiabetic control	10	446 ± 8		7.9 ± 0.5
Nondiabetic + salbutamol	10		471 ± 6	7.3 ± 0.5
Diabetic control	10	452 ± 6	333 ± 14	42.4 ± 1.6
Diabetic + doxazosin	10	474 ± 7	311 ± 10	39.9 ± 1.5
Diabetic + doxazosin + N^G-nitro-L-arginine	8	471 ± 5	324 ± 12	45.9 ± 1.6
Diabetic + salbutamol	10	464 ± 5	338 ± 6	40.6 ± 1.5
Diabetic + salbutamol + N^G-nitro-L-arginine	9	459 ± 6	346 ± 9	43.6 ± 1.1

Data are mean ± S.E.M.

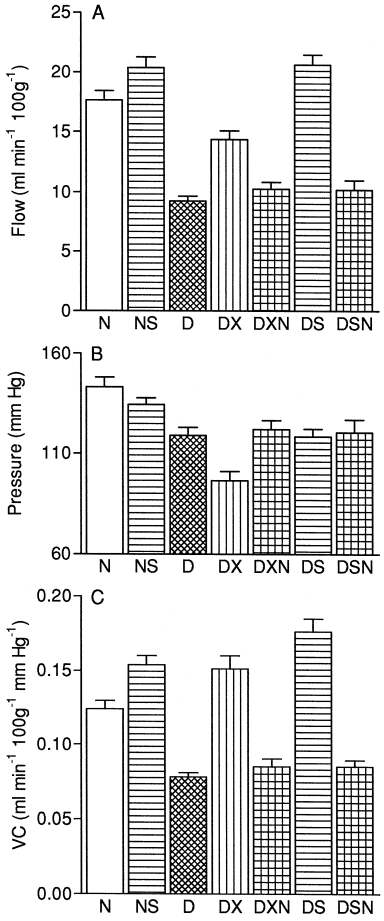

Fig. 2. Sciatic nerve nutritive perfusion parameters; (A) endoneurial nutritive blood flow, (B) mean systemic blood pressure, and (C) nutritive vascular conductance (VC). N, nondiabetic control group; NS nondiabetic group treated with salbutamol (0.3 mg kg^{-1} day^{-1}) for 2 weeks; D, 8-week diabetic control group; DX, 8-week diabetic group treated for the last 2 weeks with doxazosin (10 mg kg^{-1} day^{-1}); DXN, 8-week diabetic group treated for the last 2 weeks with both doxazosin and N^G-nitro-L-arginine (10 mg kg^{-1} day^{-1}); DS, 8-week diabetic group treated for the last 2 weeks with salbutamol; DSN, 8-week diabetic group treated for the last 2 weeks with both salbutamol and N^G-nitro-L-arginine. Group $n=$ 8–10. Error bars are S.E.M.

lar conductance (Fig. 2C). There was a $37.3 \pm 2.4\%$ ($P < 0.001$) reduction in conductance with untreated diabetes which was more than completely corrected by doxazosin and salbutamol treatments ($P < 0.001$), the resultant values being supernormal by $21.9 \pm 6.9\%$ ($P < 0.01$) and $42.0 \pm 7.0\%$ ($P < 0.001$), respectively. In salbutamol-treated nondiabetic rats, conductance was also significantly increased ($24.0 \pm 5.0\%$, $P < 0.01$) compared to the nondiabetic control group. N^G-Nitro-L-arginine co-treatment of doxazosin or salbutamol-treated diabetic rats reduced endoneurial vascular conductance ($P < 0.001$) to values not significantly different from those for the untreated diabetic group. When data from treated diabetic groups were pooled, conduction velocity as the dependent variable was strongly correlated with nutritive blood flow ($r^2 = 0.52$, $P < 0.0001$) and conductance ($r^2 = 0.68$, $P < 0.0001$).

Hydrogen clearance curves for peripheral nerve are usually composed of two components. A fast component arises due to clearance by large vessels (non-nutritive arterial, venous and particularly arteriovenous flow) and a slow nutritive component results from capillary clearance (Low et al., 1989). The composite of these components specifies total endoneurial blood flow (Fig. 3A). This was $60.8 \pm 4.4\%$ ($P < 0.01$) reduced by diabetes. The deficit was corrected by salbutamol treatment ($81.8 \pm 16.3\%$, $P < 0.01$ versus diabetic control group, NS versus nondiabetic control group); this protection was completely abolished by co-treatment with N^G-nitro-L-arginine ($P < 0.001$) such that the resulting value was only $56.8 \pm 6.6\%$

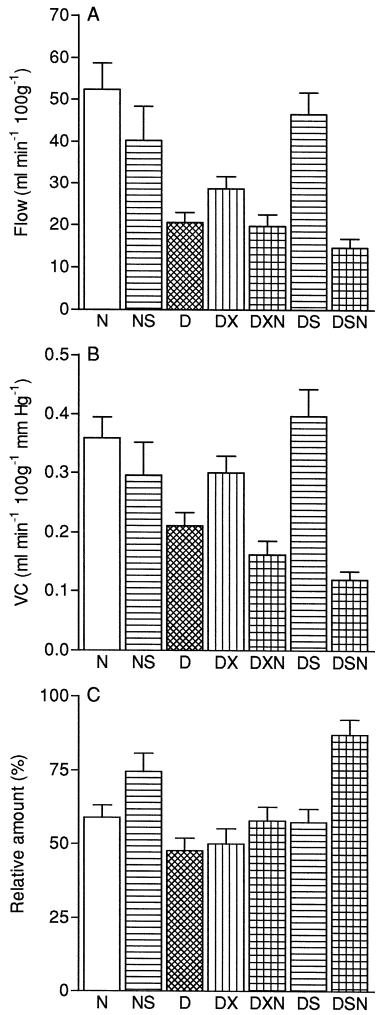

Fig. 3. Sciatic nerve total endoneurial perfusion parameters; (A) composite blood flow, (B) composite vascular conductance (VC) and (C) the relative nutritive hydrogen clearance. N, nondiabetic control group; NS nondiabetic group treated with salbutamol (0.3 mg kg^{-1} day^{-1}) for 2 weeks; D, 8-week diabetic control group; DX, 8-week diabetic group treated for the last 2 weeks with doxazosin (10 mg kg^{-1} day^{-1}); DXN, 8-week diabetic group treated for the last 2 weeks with both doxazosin and N^G-nitro-L-arginine (10 mg kg^{-1} day^{-1}); DS, 8-week diabetic group treated for the last 2 weeks with salbutamol; DSN, 8-week diabetic group treated for the last 2 weeks with both salbutamol and N^G-nitro-L-arginine. Group $n = 8–10$. Error bars are S.E.M.

($P < 0.01$) of that for the diabetic control group. Doxazosin treatment gave a lesser correction ($25.5 \pm 9.4\%$) of the diabetic deficit which was not statistically significant; composite flow remained reduced compared to the nondiabetic control group ($P < 0.01$). Joint doxazosin and N^G-nitro-L-arginine treatment resulted in flow values within the diabetic control range. When the composite flow data were adjusted to take account of systemic blood pressure variations, a $41.2 \pm 6.1\%$ ($P < 0.01$) diabetic deficit in vascular conductance remained (Fig. 3B). This was completely corrected by salbutamol ($P < 0.01$) treatment whereas with doxazosin, conductance was intermediate between diabetic and nondiabetic control values, not significantly different from either group. Salbutamol treatment did not significantly alter composite flow or conductance in nondiabetic rats. The proportion of hydrogen clearance by the nutritive component (Fig. 3C) was not significantly affected by diabetes or treatment, except in the joint salbutamol and N^G-nitro-L-arginine treatment group, where nutritive flow predominated compared to nondiabetic control and all other diabetic groups ($P < 0.01$). There may have been a tendency for an increase in the proportion of nutritive flow in the salbutamol treated nondiabetic group, however, this did not reach statistical significance.

4. Discussion

The data show that diabetes-induced deficits in motor conduction velocity can be corrected by an α_1-adrenoceptor antagonist and a β_2-adrenoceptor agonist. The α_1-adrenoceptor-mediated effect is in agreement with a previous study using prazosin (Cameron et al., 1991a), or using a less specific adrenoceptor blockade by the antagonist, carvedilol (Cotter and Cameron, 1995), or with chemical sympathectomy by guanethidine (Cameron et al., 1991b). Correction of reduced nerve conduction velocity by β_2-adrenoceptor agonist treatment in diabetic rats has not previously been described. Doxazosin and salbutamol also increased nutritive endoneurial blood flow, further supporting the hypothesis that reduced perfusion is a major determinant of conduction velocity defects in diabetes (Low et al., 1989; Cameron and Cotter, 1994; Tesfaye et al., 1994).

Previous investigations have stressed the importance of α-adrenoceptor-mediated vasoconstriction of vasa nervorum epineurial vessels under the influence of sympathetic fibres in nerve blood flow control (Appenzeller et al., 1984; Kihara and Low, 1990; Cameron et al., 1991b). However, the data for salbutamol treatment suggest that vasa nervorum also has β_2-adrenoceptors in diabetic and nondiabetic rats, although their physiological role in the control of blood flow is not known. β-Adrenoceptor stimulation causes vasodilation in arterioles supplying several tissues including abdominal viscera, heart, skeletal muscles and brain (Belfrage, 1978; Lass et al., 1989; Gardiner et al., 1991; Randall and McCulloch, 1995). Experiments using a similar diabetic model and methodology showed that carvedilol treatment provided a high level of neuroprotection and markedly increased sciatic nerve blood flow (Cotter and Cameron, 1995). Carvedilol, is an α_1-adrenoceptor antagonist but also blocks β_1- and β_2-adrenoceptors (Willette et al., 1990). The benefits of α_1-adrenoceptor blockade could potentially have been limited by the opposing effect of β_2-adrenoceptor antagonism if tonic β_2-adrenoceptor-mediated activity was important for the maintenance of resting blood flow. As carvedilol markedly increased blood flow in both nondiabetic and diabetic rats, it may be that β_2-adrenoceptor activation is an intermittent rather than a tonic phenomenon under normal physiological conditions in conscious rats. General activation of the sympathetic system, for example in anticipation of and during intense physical activity, causes adrenaline release from adrenal medulla. In turn, this stimulates β_2-adrenoceptors on skeletal muscle vessels, which increases muscle perfusion (Hudlicka, 1985). It is plausible that a similar mechanism exists in peripheral nerve during sympathetic activation. Stimulation of vasa nervorum β_2-adrenoceptors by adrenaline may have two physiological advantages. First it would oppose any α_1-adrenoceptor-mediated vasoconstriction resulting from increased sympathetic nerve activity and noradrenaline release. Second, it could increase nerve blood flow in anticipation of increased energy demands necessary to sustain the elevated motor and sensory action potential traffic that would occur with intense physical activity.

Co-treatment with N^G-nitro-L-arginine markedly attenuated the conduction velocity effects of doxazosin and salbutamol. There was a parallel reduction in nutritive endoneurial blood flow, which further supports the link between impaired perfusion and conduction velocity. This is in agreement with the effects of nitric oxide synthase inhibition on treatments that target metabolic changes in diabetic rats, such as aldose reductase inhibition, *n*-6 essential fatty acids, antioxidants, aminoguanidine and L-carnitine, which also increase nerve blood flow and conduction velocity (Cameron and Cotter, 1995, 1996a, 1997; Cameron et al., 1996). It is unlikely that N^G-nitro-L-arginine had a direct effect on the large myelinated fibres that dominate conduction velocity measurements because they do not contain neuronal nitric oxide synthase (Yagihashi, 1995). Thus, the data emphasise the importance of nitric oxide in nerve blood flow control mechanisms, even in diabetic rats where vasa nervorum agonist-stimulated endothelial nitric oxide synthesis or action is reduced (Kihara and Low, 1995; Maxfield et al., 1997).

The improvement in sciatic nutritive blood flow by doxazosin and salbutamol treatment in diabetic rats was profoundly depressed by nitric oxide synthase inhibition. Doxazosin blocks vascular smooth muscle α_1-adrenoceptors, an action that should not be directly opposed by N^G-nitro-L-arginine which acts on the endothelium. One

possible explanation is that endothelial nitric oxide release is stimulated by flow (Fujii et al., 1992). Thus, the increased flow caused by α_1-adrenoceptor blockade would normally be further reinforced by flow-induced nitric oxide mediated vasodilation; an action abolished by N^G-nitro-L-arginine, thereby diminishing the apparent effect of doxazosin. Moreover, vasa nervorum prostacyclin-mediated vasodilation is reduced by diabetes as a result of impaired *n*-6 essential fatty acid metabolism (Ward et al., 1989). This would enhance N^G-nitro-L-arginine's effect by limiting the interaction between prostanoid and nitric oxide systems and the possibility of compensatory flow induced prostacyclin release (Cameron et al., 1996). In addition, activity in other vasoconstrictor systems is increased by diabetes and nitric oxide blockade would further enhance vasoreactivity to them. Thus, angiotensin II and endothelin systems exert greater effects on vasa nervorum in diabetic than in nondiabetic rats (Maxfield et al., 1993, 1995; Cameron et al., 1994; Cameron and Cotter, 1996a,b). The importance of non-adrenergic mechanisms to the diabetic nerve blood flow deficit is supported by findings for epi-perineurial vessels, which are the main controlling elements of vasa nervorum (Kihara and Low, 1990). Thus, when these vessels are suffused with a dose of noradrenaline causing maximal vasoconstriction, a diabetic flow deficit remains which, in percentage terms, is of similar magnitude to the resting deficit (Maxfield et al., 1997). This is also true in the presence of a high dose of nitric oxide synthase inhibitor, therefore non-nitrergic non-adrenergic mechanisms must be responsible. Thus, while the data show that α_1-adrenoceptor-mediated vasoconstriction makes an important contribution to the blood flow deficit in diabetic rats, nonetheless, the degree of vasodilation produced by doxazosin is insufficient to overcome the effects of other vasoconstrictor systems when reactivity to them is enhanced by nitric oxide synthase inhibition. β_2-Adrenoceptor-mediated vasodilation by salbutamol was also insufficient to overcome the effects of N^G-nitro-L-arginine in diabetic rats, presumably for similar reasons. Because N^G-nitro-L-arginine co-treatment had similar consequences with salbutamol and doxazosin, it is not possible to elucidate from this data whether vasa nervorum β_2-adrenoceptor-mediated vasodilation depends only on a vascular smooth muscle effect, or also involves direct receptor-mediated stimulation of endothelial nitric oxide production by salbutamol.

One potential advantage of salbutamol over doxazosin treatment is the lack of a hypotensive response, presumably because β_2-adrenoceptors have a more restricted distribution than α_1-adrenoceptors in the general vasculature. The data for doxazosin show that hypotension is directly reflected in reduced nutritive and composite blood flow, because of the minimal pressure autoregulation capacity of vasa nervorum (Low et al., 1989). Salbutamol completely and doxazosin partially restored diabetic deficits in composite flow and vascular conductance, suggesting that both receptor types can participate in the control of nutritive and non-nutritive flow. However, the data do indicate that vascular responses to the blockade of α_1-adrenoceptors or stimulation of β_2-adrenoceptors may differ subtly. In combination with N^G-nitro-L-arginine, the proportion of hydrogen cleared by nutritive flow was increased with salbutamol. In contrast, doxazosin treatment did not have this effect. Thus, there may be greater β_2-adrenoceptor than α_1-adrenoceptor involvement in the vascular elements directing flow to the endoneurial capillary bed, as opposed to through arteriovenous shunts. This becomes particularly apparent with blanket suppression of all flow components by nitric oxide synthase inhibition (Cameron et al., 1996).

In conclusion, the data stress the importance of impaired blood flow in the aetiology of experimental diabetic neuropathy and highlight the permissive role of vasa nervorum nitric oxide in modulating responses to vasodilators. The study also shows that β_2-adrenoceptor stimulation markedly increases endoneurial blood flow, sufficient to correct nerve dysfunction in diabetic rats. Thus, β_2-adrenoceptor agonists could be candidates for clinical neuropathy trials and it is possible that any effects may be enhanced by co-treatments targeting the metabolic changes responsible for the impaired endothelial nitric oxide system in diabetes.

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