



Aminoguanidine - effects on endoneurial vasoactive nitric oxide and on motor nerve conduction velocity in control and streptozotocin-diabetic rats

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1 The effects of aminoguanidine (AG) treatment on reductions in motor nerve conduction velocity (MNCV) and sciatic nerve blood flow, indexed by laser Doppler flux (LDF), were investigated in rats with experimental diabetes (streptozotocin-induced; 8–10 weeks duration). The contribution of endoneurial vasoactive nitric oxide to the LDF of these animals was also investigated by the direct micro-injection of N^G-nitro-L-arginine methyl ester (L-NAME; 1 nmol in 1 μ l), followed by L-arginine (100 nmol in 1 μ l), into the sciatic nerve endoneurium.

2 The MNCV (m s⁻¹, mean \pm 1 s.d.) of diabetic rats (38.2 \pm 1.5) was lower ($P < 0.01$) than that of age-matched controls (47.2 \pm 4.2). AG treatment (50 mg kg⁻¹ day⁻¹, i.p.) attenuated the diabetes-induced deficits in MNCV (43.4 \pm 5.9; $P < 0.01$), but had no effect in controls (48.8 \pm 3.8) or, if administered via drinking water (1 g l⁻¹), diabetics (37.4 \pm 4.1).

3 L-NAME markedly reduced the resting LDF (arbitrary units; mean \pm s.e.mean) of controls (209 \pm 13 to 120 \pm 18; $P < 0.005$), an effect reversed by subsequent L-arginine (to 206 \pm 27). In diabetic rats the LDF reduction following L-NAME was much smaller (111 \pm 11 to 84 \pm 6; $P < 0.05$), but the change with L-arginine was significantly increased (to 145 \pm 12; $P < 0.001$).

4 AG treatment increased the resting LDF of control (265 \pm 34) and diabetic rats (133 \pm 14 for daily injection and 119 \pm 13 for drinking water). The responses to L-NAME and L-arginine were not changed markedly by AG treatment. However, L-arginine appeared to be less effective.

5 In conclusion, these data suggest that AG treatment may affect nitric oxide production in the *vasa nervorum* of peripheral nerves. However, the effects of AG-treatment are not consistent with the prevention of a diabetes-associated reduction in endoneurial nitric oxide production. The mechanisms by which AG attenuates nerve conduction slowing in streptozotocin-diabetic rats therefore remain unclear.

Keywords: Aminoguanidine; diabetic neuropathy; streptozotocin; diabetes mellitus; nitric oxide; nerve conduction; velocity; blood flow; laser Doppler; L-NAME; L-arginine

Introduction

Diabetic neuropathy is a degenerative complication characterized by structural and functional abnormalities in peripheral nerves (for review see Thomas & Tomlinson, 1992). Recently, endoneurial hypoxia as a consequence of peripheral nerve ischaemia has been proposed as a contributory factor in the pathogenesis of diabetic neuropathy (for review see Tesfaye *et al.*, 1994). In rats, the induction of experimental diabetes leads to reductions in sciatic nerve blood flow and endoneurial oxygen tensions (Tuck *et al.*, 1984). Moreover, these changes parallel reductions in nerve conduction velocity (Stevens & Tomlinson, 1995), an early electrophysiological abnormality observed in experimental and human diabetes mellitus. Peripheral nerve blood flow has also been shown to be reduced in man (Tefsaye *et al.*, 1994) and rats with genetic diabetes mellitus (Stevens *et al.*, 1994a).

Although the causes of endoneurial ischaemia remain unresolved, it is plausible that a localized defective production or release of locally acting vasoreactive substances may be responsible (Tefsaye *et al.*, 1994; Poston & Taylor, 1995). In diabetic rats, increased free-radical activity has been shown to attenuate endothelium-dependent relaxation of isolated aortae (Pieper & Garrett, 1988; Hattori *et al.*, 1991), a process which may also occur in the sciatic nerves of such animals (Low & Nickander, 1991). The release of other mediators such as the vasodilator prostacyclin has also been shown to be reduced in peripheral nerves from experimentally diabetic rats (Ward *et al.*, 1989; Stevens *et al.*, 1993). The defective

release of such mediators may have important consequences in the regulation of peripheral nerve blood flow and thus diabetic neuropathy.

Recently we have demonstrated the susceptibility of peripheral nerve blood flow, as indexed by the real-time monitoring of sciatic nerve laser Doppler flux (LDF), to drugs administered via direct microinjection into the sciatic nerve endoneurium (Omawari *et al.*, 1996). With this technique the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) followed by L-arginine was shown to cause sciatic nerve LDF to be decreased and increased, respectively. Neither saline nor D-NAME, which does not inhibit nitric oxide production (Graves & Poston, 1993), affected sciatic nerve LDF. In streptozotocin (STZ)-diabetic rats the effects of L-NAME were markedly attenuated, whereas the effects of L-arginine were augmented, implicating a deficiency in the local production of nitric oxide.

Aminoguanidine (AG) can prevent the development of motor and sensory nerve conduction velocity deficits in STZ-diabetic rats (Cameron *et al.*, 1992; Yagihashi *et al.*, 1992), possibly by an effect upon nerve blood flow. Kihara *et al.* (1991) found that AG treatment prevented the peripheral nerve ischaemia associated with experimental diabetes; they hypothesized that this was due to an action of AG on the microvasculature of the nerve (Kihara *et al.*, 1991). This may be mediated via the prevention of advanced glycosylation end product formation in blood vessel walls (Brownlee *et al.*, 1986; Hill & Ege, 1994), the inhibition of an isoform of nitric oxide synthase (Tilton *et al.*, 1993) or the enhanced release of vasodilator substances such as prostacyclin (Dewhurst *et al.*, 1994) in the *vasa nervorum*.

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Therefore in this study we investigated the effects of AG treatment in control and STZ-diabetic rats, examining effects upon sciatic nerve LDF and motor nerve conduction velocity (MNCV). The technique of sciatic nerve microinjection was also used to elucidate the possible effects of AG on the contribution of endoneurial nitric oxide production to sciatic nerve LDF.

Methods

Induction of diabetes and treatments

Male Wistar rats, weight 280–320 g (Charles River, U.K.), were randomly assigned to five groups – two age-matched groups and three diabetic groups. Control and diabetic groups remained untreated or received daily injections of AG (50 mg kg⁻¹, i.p.). The third diabetic group was treated with AG, administered via drinking water (1 g l⁻¹). Following an overnight fast, diabetes was induced by a single injection of streptozotocin (55 mg kg⁻¹ body weight, i.p.), freshly dissolved in sterile saline (0.9% w/v). Thereafter all groups were allowed free access to standard laboratory chow (Rat & Mouse No. 1 maintenance diet, Special Diets Services, U.K.) and water, the second group being used as age-matched controls. Three days later diabetes was confirmed by reflectance photometry of tail vein blood glucose (Reflolux, Boehringer Mannheim, Germany), and all treatments begun and continued until the end of the protocol (8–10 weeks). Rats were accepted as diabetic if blood glucose concentrations exceeded 15 mM.

Measurement of cardiovascular variables and sciatic nerve laser Doppler flux

Anaesthesia was induced with halothane and cannulae inserted into the jugular vein, for the infusion of anaesthetic, and carotid artery for the monitoring of arterial blood pressures via a pressure transducer (Type 4-327 L221; Bell and Howell). Anaesthesia was maintained by a bolus of diazepam (3 mg kg⁻¹, i.v., 0.5% w/v; Diazemulus, Dumex, U.K.) and pentobarbitone sodium (approximately 13 mg kg⁻¹, 3% w/v; Sagatal, Rhône Merieux, U.K.), with extra anaesthetic mixture (1.25 mg ml⁻¹ and 15 mg ml⁻¹, respectively) administered as required. Throughout all experimental procedures body temperature was maintained at 37°C via a rectal temperature probe connected to a heated homeothermic mat. The left sciatic nerve was exposed below the sciatic notch by careful separation and displacement of overlying muscle layers under a dissecting microscope. A standard optic laser probe (tip diameter 0.8 mm) was placed at the nerve surface and LDF monitored by a laser Doppler flow monitor (frequency 15 kHz, 810 nm, power 1.3 mA; Moor Instruments, U.K. Type MBF3D) at a sampling rate of 40 Hz and time constant of 1.00 s. Carotid arterial pressure and laser Doppler signals were digitized (MacLab/8 Interface Module; AD Instruments Ltd, U.K.) and recorded (100 Hz) by use of Chart version 3.4.3 software (AD Instruments) running on a Macintosh IIsi personal computer. These variables were continuously recorded until the effects of the final (L-arginine) sciatic nerve microinjection had reached a plateau (see below). Nerve Doppler flux values were obtained by averaging the data recorded over 4 min periods (i.e. over entire basal periods or once the effects of a microinjection had formed a plateau). The reduced laser probe tip diameter explains the numerically smaller values for LDF in this study compared with our earlier publications (see above). LDF values are presented in arbitrary units (arb.u).

Sciatic nerve micro-injections

Drugs were injected into the sciatic nerve endoneurium via a glass micropipette. Following exposure of the sciatic nerve, a small hole (20–30 µm) was made through the epineurium by a

tungsten microelectrode (shaft diameter 250 µm with 12° taper to tip), allowing access to the endoneurium. A glass micropipette (tip diameter 10–15 µm) was thus advanced into the endoneurium with an X-Y-Z micropositioner. A Drummond microdispenser (Laser Labs, Southampton, U.K.) connected to this pipette was used to administer µl volumes of drug to the endoneurium. Sciatic nerve LDF was monitored 1 to 2 mm distal to the micropipette tip. In some rats, D-NAME (1 µl) was injected to demonstrate that the physical effect of injection was insignificant. Following stabilization of arterial pressure a 4 min basal period was recorded before the injection of L-NAME (1 nmol in 1 µl) over a 5 min period. Once the effects of L-NAME had formed a plateau, the micropipette containing L-NAME was exchanged for one containing L-arginine. Another basal period (4 min) was recorded and L-arginine (100 nmol in 1 µl) then microinjected, again over a 5 min period, and recording of LDF and cardiovascular variables continued as before.

Motor nerve conduction velocity

MNCV was measured in sciatic nerves contralateral to those receiving microinjections. This was done after the measurement of nerve blood flow as to eliminate the possible effects of electrical stimulation on nerve blood flow (Omawari *et al.*, 1996). The temperature adjacent to the mid-femoral sciatic nerve was monitored with a microthermocouple connected to an electronic thermometer (Comark, U.K.), and was maintained at 37°C by an infra-red lamp. The sciatic nerve was stimulated with a single constant current (10 mA) square wave pulse (duration 100 µs), firstly at the sciatic notch and then at the Achilles tendon. Pulses were delivered via monopolar needle electrodes. Electromyograms were recorded by needle electrodes inserted into the interosseous muscle and skin of the foot, amplified 100 fold, digitised (MacLab/8, see above) and recorded with 'Scope v3.4' software running on a Macintosh IIsi personal computer. The temporal separations of the electromyograms were used to determine nerve conduction velocity as described previously (Karasu *et al.*, 1995). At the end of all measurements blood was collected for plasma glucose measurement by a glucose oxidase assay kit, as defined previously (Karasu *et al.*, 1995).

Statistical analysis

All data are presented as means ± 1 s.d. For clarity data in Figure 1 and Table 3 are presented as means ± s.e.mean. Acute drug effects were evaluated by comparison within animal (effect versus baseline) by use of paired *t* tests. Single comparisons between groups used one-way analysis of variance with Duncan's multiple range tests (*P* < 0.01 and *P* < 0.05) where *P* < 0.05 and there was homogeneity of variance (Levene's test *P* < 0.05).

Drugs

All drugs were obtained from Sigma Chemical Company (U.K.) unless indicated otherwise in Methods section.

Results

Body weight and plasma glucose

Data showing final plasma glucose concentrations and changes in body weight for all groups are summarized in Table 1. Diabetic rats were hyperglycaemic and of lower body weight than age-matched controls. AG treatment significantly increased the final body weights of control and diabetic rats when administered via intra-peritoneal injection, but was without effect when administered via drinking water. AG treatment had no effect upon final plasma glucose concentrations.

Effects of aminoguanidine treatment on motor nerve conduction velocity

Diabetic animals had significantly reduced motor nerve conduction velocities compared with those of untreated age-matched controls (Table 1). AG treatment did not affect MNCV in control animals, but attenuated the diabetes-induced reduction in MNCV if administered by daily intraperitoneal injection. The nerve conduction velocity of diabetic rats treated with AG via drinking water was similar to that of untreated diabetic animals (Table 1).

Effects of aminoguanidine treatment on arterial pressure and sciatic nerve laser Doppler flux

Diabetic animals exhibited a minor degree of hypotension relative to non-diabetic control animals, which was significant for the untreated diabetic group (Table 2). On a few occasions the microinjection of drugs into the sciatic nerve endoneurium caused a modest, transient (<2 min) change in mean arterial pressure (see Omawari *et al.*, 1996 for traces). However, the changes in LDF elicited by L-NAME or L-arginine were not associated with changes in arterial pressures. Mean arterial

pressures for the time periods over which LDF data were obtained are summarized in Table 2. Arterial pressures measured before and after the microinjection of drugs into the sciatic nerve endoneurium were not significantly different. Bradycardia, characteristic of experimentally diabetic rats, was also observed in diabetic groups and was also unaffected by endoneurial microinjections (Table 2).

The resting sciatic nerve LDF was significantly lower in diabetic rats ($P < 0.01$) compared to that of untreated controls (Table 3). Treatment with AG increased resting sciatic nerve LDF in controls, though this effect was variable between animals and thus not significant. AG treatment had no significant effect on nerve LDF in diabetic rats, whether given by intraperitoneal injection or via drinking water.

Modulation of sciatic nerve Doppler flux by L-NAME and L-arginine

The microinjection of L-NAME into the sciatic nerve endoneurium of untreated and AG-treated control rats significantly reduced nerve LDF (Figure 1 and Table 3). The subsequent microinjection of L-arginine reversed the effects of L-NAME, restoring the final nerve LDF of the untreated

Table 1 Body weight, plasma glucose and motor nerve conduction velocity data for experimental groups

Group	n	Body weight (g)		Plasma glucose (mM)	MNCV (m s ⁻¹)
		Initial	Final		
Control rats					
Untreated	8	307.8 ± 14.8	525.4 ± 43.7 ^a	6.76 ± 0.53 ^c	47.2 ± 4.2 ^a
AG-treated (i.p.)	10	322.8 ± 9.7	572.6 ± 49.2 ^b	6.31 ± 1.10 ^c	48.8 ± 3.8 ^a
Diabetic rats					
Untreated	9	318.6 ± 13.8	276.1 ± 30.7 ^c	35.28 ± 3.71 ^d	38.2 ± 1.5 ^c
AG-treated (i.p.)	11	316.2 ± 13.5	333.5 ± 32.1 ^d	33.10 ± 3.75 ^d	43.4 ± 5.9 ^d
AG-treated (d.w.)	9	311.3 ± 7.4	291.6 ± 48.4 ^e	32.45 ± 2.12 ^d	37.4 ± 4.1 ^c

Data are mean ± 1 s.d. and were analysed by one-way ANOVA with Duncan's multiple range tests. Superscripts denote significant differences between groups within columns at $P < 0.05$ a vs b and d vs e and at $P < 0.01$ a or b vs c, d or e and c vs d. Abbreviations—AG, aminoguanidine; MNCV, motor nerve conduction velocity and d.w., drinking water.

Table 2 Effects of endoneurial microinjection on mean arterial pressures and resting heart rates of experimental groups

Group	Resting heart rate (beats min ⁻¹)	Mean arterial pressure (mmHg)		
		Initial	L-NAME	L-Arginine
Control rats				
Untreated	436 ± 33 ^c	102 ± 3 ^a	100 ± 7	106 ± 3
AG-treated (i.p.)	404 ± 20 ^c	102 ± 5 ^a	102 ± 7	109 ± 6
Diabetic rats				
Untreated	336 ± 21 ^d	95 ± 3 ^b	95 ± 5	96 ± 3
AG-treated (i.p.)	353 ± 24 ^d	97 ± 6	99 ± 8	97 ± 6
AG-treated (d.w.)	349 ± 43 ^d	99 ± 6	102 ± 13	102 ± 9

Data are mean ± 1 s.d. and were analysed by one-way ANOVA with Duncan's multiple range tests. Superscripts denote significant differences between groups within columns at $P < 0.05$ a vs. b and at $P < 0.01$ c vs. d. Typical differences between initial arterial pressures shown only. Data for mean arterial pressures were obtained over time periods concomitant with those for LDF. Abbreviations—AG, aminoguanidine and d.w., drinking water.

Table 3 Effects of N^G-nitro-L-arginine methyl ester (L-NAME) and L-arginine on sciatic nerve laser Doppler flux

Group	Sciatic nerve laser Doppler flux (arbitrary units)					
	Pre-L-NAME	Post-L-NAME	% fall	Pre-L-arginine	Post-L-arginine	% rise
Control rats						
Untreated	209 ± 13	120 ± 18	43 ± 8	133 ± 26	206 ± 27	66 ± 28
AG-treated (i.p.)	265 ± 34	171 ± 18	28 ± 12	163 ± 19	196 ± 41	16 ± 13
Diabetic rats						
Untreated	111 ± 11 ^a	84 ± 6	21 ± 7	83 ± 7	145 ± 12 ^b	76 ± 11
AG-treated (i.p.)	133 ± 14	106 ± 15	17 ± 10	96 ± 13	127 ± 22	30 ± 8
AG-treated (d.w.)	119 ± 13	84 ± 8	25 ± 8	88 ± 16	123 ± 8	56 ± 17

Data are mean ± 1 s.e.mean and were analysed by paired *t* tests. Superscripts denote that the sciatic nerve laser Doppler flux following both microinjections was significantly different from initial resting values at $P < 0.012$ a vs. b. Significant changes following individual drug microinjections are indicated in Figure 1. Abbreviations—AG, aminoguanidine and d.w., drinking water.

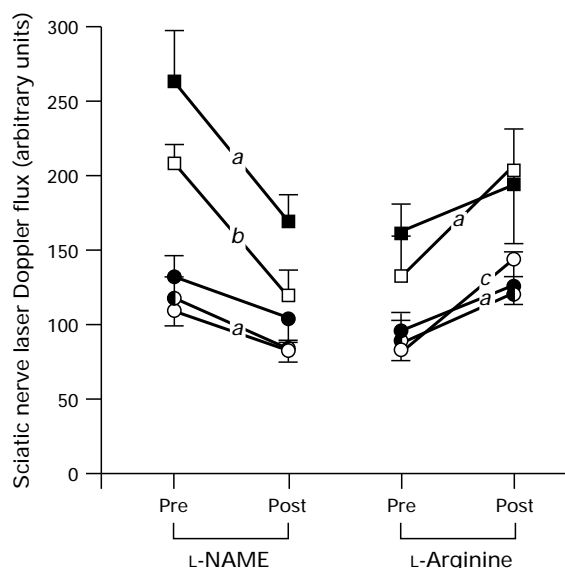


Figure 1 Effects of N^G-nitro-L-arginine methyl ester (L-NAME; 1 nmol in 1 μ l) and L-arginine (100 nmol in 1 μ l) on sciatic nerve laser Doppler flux of control (□, ■) and streptozotocin-diabetic rats (○, ●). Rats remained untreated (□, ○) or received aminoguanidine, either by daily intraperitoneal injection (50 mg kg⁻¹, ■, ●), or in drinking water (1 g l⁻¹, ○, ● for diabetic only). Data are mean with vertical lines showing s.e.mean, and were analysed by paired *t* tests. Significant differences after microinjection of L-NAME or L-arginine are as indicated at *P* < 0.05 (a), *P* < 0.005 (b) and *P* < 0.001 (c). Unmarked lines indicate not significant.

control group to $95 \pm 22\%$ of its initial value (mean \pm s.d.), and for the AG-treated control group to $79 \pm 38\%$ of its initial value. The sciatic nerve LDF of diabetic rats was also reduced by the microinjection of L-NAME, though in these animals the effect of L-NAME was less marked (Figure 1 and Table 3). For the untreated diabetic group the subsequent microinjection of L-arginine increased the nerve LDF to levels which were significantly (*P* < 0.012) greater than initial resting values ($136 \pm 31\%$ of initial; Table 3). In contrast, L-arginine increased the nerve LDF of AG-treated diabetic rats to levels which were not significantly different from initial resting values ($98 \pm 38\%$ for intraperitoneal and $114 \pm 44\%$ for drinking water). As shown previously (Omawari *et al.*, 1996) D-NAME, which does not inhibit nitric oxide production (Graves & Poston, 1993), had no effect on sciatic nerve LDF (data not shown).

Discussion

This study has confirmed the development of a nerve Doppler flux deficit and motor nerve conduction slowing in STZ-diabetic rats, findings which have been well documented in rats with both chemical and genetic forms of diabetes (see Introduction). In addition this study confirmed our previous finding (Omawari *et al.*, 1996) that L-NAME, microinjected directly into the sciatic nerve endoneurium, causes smaller reductions in the nerve LDF of diabetic rats than in age-matched controls. Similar reductions in nerve blood flow were also observed in a recent study by Kihara and Low (1995), though in their study the topical application of two different nitric oxide synthase (NOS) inhibitors was associated with a moderate increase in nerve blood flow before its reduction. The time required for the NOS inhibitors to reduce nerve blood flow was also approximately twice that observed in this study. These minor discrepancies are probably explainable by methodological differences. In the study by Kihara and Low (1995) the nitric oxide synthase inhibitors were administered to the

sciatic nerve topically, via addition to a pool of physiological buffer contained within surrounding muscle layers. Thus, it is possible that in their study the initial increase in nerve blood flow was mediated by actions of the NOS inhibitors on vascular tissues other than the sciatic nerve endoneurium, principally local skeletal muscle, and the slower onset of effect due to the increased drug diffusion distances. Regardless of these differences, the NOS inhibitors reduced the nerve blood flow of control rats to a similar extent in both studies (L-NMMA reduced nerve blood flow by 38% in their study and L-NAME by 43% in this study). Another common finding of these studies was the reduced capacity of NOS inhibitors to decrease the sciatic nerve blood flow of diabetic rats. These studies not only indicate a powerful role for nitric oxide in the maintenance of sciatic nerve blood flow, but suggest that nitric oxide production in the resting nerve of diabetic rats is reduced (see Omawari *et al.*, 1996).

In examining the effects of L-NAME on endoneurial nitric oxide production it is important to bear in mind that other factors, such as a reduction in the production of vasodilator prostanoids are also likely to contribute (Stevens *et al.*, 1993; Omawari *et al.*, 1996). This is indicated by the fact that neither L-arginine nor sodium nitroprusside are capable of completely restoring nerve LDF to levels observed in non-diabetic animals (this study and Omawari *et al.*, 1996). Non-diabetic rats treated with low doses of cyclo-oxygenase and NOS inhibitors in combination have also been shown to develop nerve conduction velocities equivalent to those observed in diabetic rats of the same duration (Cameron *et al.*, 1993). However, it is possible that the failure of sodium nitroprusside or L-arginine completely to restore nerve LDF reflects an acutely irreversible modification to the structure of the *vasa nervorum* which limited vasodilatation. Micro-vascular abnormalities such as increased basement membrane thickening and reduced endoneurial capillary density are clearly observed in the *vasa nervorum* of experimentally diabetic rats (Yagihashi *et al.*, 1979; Powell *et al.*, 1980; Cameron *et al.*, 1991).

AG treatment had no effect upon the systemic arterial pressure of control or STZ-diabetic rats, an observation found by others (Tilton *et al.*, 1993). However, it is possible that with long-term AG treatment cardiovascular and baroregulatory reflexes compensate for a minor effect on peripheral vascular tone, such that the measurements made here would not detect it. In fact, studies suggest that similar doses of AG to those used in this study can produce minor elevations in systemic arterial pressure (Gardiner *et al.*, 1996). Nonetheless, AG is known to be a relatively selective inhibitor of the cytokine-inducible isoform of NOS (Laszlo *et al.*, 1995; Wu *et al.*, 1995) and these data suggest, therefore, that constitutive endothelial NOS is the key enzyme responsible for vasoactive nitric oxide production in the peripheral vasculature of diabetic rats. It is also important to note that arterial pressures concomitant to the recording of nerve LDF were not different before and after the endoneurial microinjections. Sciatic nerve blood flow has a limited capacity for autoregulation and acute changes in arterial pressures can affect nerve blood flow (Low & Tuck, 1984; Takeuchi & Low, 1987). The local microinjection of drugs directly into the sciatic nerve endoneurium avoids systemic influences of drugs upon nerve blood flow, such as the hypertensive effects of L-NAME.

In disagreement with a study by Kihara *et al.* (1991), AG treatment failed to have a significant effect on the nerve Doppler flux deficit of diabetic rats. In the study of Kihara *et al.* a similar 57% reduction in sciatic nerve blood flow was observed in rats with diabetes of 8 weeks duration, but this was completely prevented by treatment with AG (50 mg kg⁻¹ day⁻¹, i.p.). Although the reasons for this discrepancy are unclear, several methodological differences may be responsible. Firstly, Kihara and co-workers used hydrogen clearance curves to measure flow within the endoneurium, a method postulated to measure nutritive flow alone (Day *et al.*, 1989). The LDF method used in our study serves only as an index of whole nerve blood flow and may not delineate effects

of AG upon epineurial and endoneurial vessels within the sciatic nerve (Stevens *et al.*, 1994a). However, sciatic nerve LDF measurements have been positively correlated to direct measurements of nerve blood flow, by use of the radiotracer iodo[¹⁴C]-antipyrine and hydrogen polarography (Rundquist *et al.*, 1985; Takeuchi & Low, 1987; Stevens *et al.*, 1994b). Clearly this matter can only be resolved by a study in which both hydrogen polarography and LDF are employed to assess the effects of AG on nerve blood flow.

It is interesting that the ability of L-arginine to reverse the effects of L-NAME was diminished in control and diabetic rats treated with AG. At present there does not appear to be an explanation for this effect in the literature, though it is possible that AG treatment reduces the intracellular availability of L-arginine for conversion into nitric oxide, or affects its uptake into the nitric oxide producing cells of the *vasa nervorum*. Clearly further studies are required to investigate the effects of AG on the bioavailability and intracellular metabolism of L-arginine before definitive conclusions can be drawn regarding how this effect is mediated.

The capacity of AG treatment to prevent reductions in the MNCV of diabetic rats has been demonstrated previously, through there are some discrepancies (Kihara *et al.*, 1991; Yagihashi *et al.*, 1992). In this study AG was ineffective when administered via drinking water, whereas Cameron *et al.* (1992) showed treatment by this route prevented diabetes related reductions in both motor and sensory nerve conduction velocities. Although there are no obvious methodological differences to account for this discrepancy, we believe that differences in the bioavailability of AG may be responsible. This is supported here by the parallel effects of AG on body weight and MNCV in diabetic rats. Thus, intraperitoneal AG increased the body weights of diabetic rats, whereas in drinking water AG had no effect. This effect of AG has been observed previously in non-diabetic rats (Baylin *et al.*, 1975). Furthermore, treating diabetic rats with doses of AG (25 mg kg⁻¹, i.p.) lower than those used in this study does not produce a significant attenuation of motor nerve conduction slowing at the duration of diabetes used in this study (Yagihashi *et al.*, 1992).

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