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Neurovascular effects of L-carnitine treatment in diabetic rats

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Abstract

The aims were to ascertain whether L-carnitine could prevent nerve blood flow and conduction deficits in 1-month diabetic rats and to examine potential neurovascular mechanisms using co-treatment with the nitric oxide synthase inhibitor, N^G-nitro-L-arginine. A 19.8% diabetic deficit in sciatic motor conduction velocity was 57.4% attenuated by L-carnitine treatment. Similarly, a 47.7% reduction in sciatic nutritive (capillary) endoneurial blood flow was 48.6% blocked by L-carnitine. Joint treatment with N^G-nitro-L-arginine completely abolished the effects on nerve conduction and nutritive flow. However, L-carnitine treatment did not alter a 50.8% diabetic deficit in total sciatic endoneurial flow, which was further depressed (61%) by N^G-nitro-L-arginine co-treatment. Thus, the effect of L-carnitine on nerve conduction in diabetic rats depends on changes in the endoneurial perfusion pattern by an action that may involve the nitric oxide system of vasa nervorum.

Keywords: Nerve conduction; Blood flow; L-Carnitine; Nitric oxide (NO); Diabetic rat

1. Introduction

Acetyl-L-carnitine has been used to treat some of the complications of experimental diabetes mellitus, such as retinopathy (Lowitt et al., 1993) and neuropathy (Cotter et al., 1995a; Ido et al., 1994; Lowitt et al., 1995). Several mechanisms have been suggested to account for the action of acetyl-L-carnitine in diabetic and non-diabetic tissues. These include improved mitchondrial lipid handling (Siliprandi et al., 1965), a neurotrophic action (Taglialatela et al., 1991, and anti-oxidant properties (Fariello and Calabrese, 1988).

The actions of L-carnitine derivatives against conduction deficits in diabetes have been suggested to be metabolic, resulting from an increase in nerve Na^+,K^+-ATPase (ATP phosphohydrolase; EC 3.6.1.3) activity (Ido et al., 1994; Stevens et al., 1995), or vascular, depending on improved endoneurial perfusion as noted using chronic acetyl- and proprionyl-L-carnitine treatment (Cotter et al., 1995a). The aim was to further elucidate the mechanisms of action; first by testing whether treatment with the L-carnitine moiety alone could prevent neurovascular dysfunction in diabetic rats. Second, by determining whether endothelial factors were important for L-carnitine's action, using co-treatment with a modest dose of a nitric oxide synthase inhibitor. Nitric oxide synthase is localised in epi- and peri-neurial vessels and is virtually absent from endoneurial nerve fibres and microvessels (Yagihashi, 1995). Thus, blockade may be used to discriminate between treatment effects on vasa nervorum and those that act 'downstream' directly on nerve fibres themselves.

2. Materials and methods

Mature male 19-week-old Sprague-Dawley rats (Aberdeen University colony) were used. Non-diabetic animals acted as onset controls, others were given streptozotocin (40–45 mg kg^{-1} freshly dissolved in sterile saline, by intraperitoneal injection). Diabetes was verified 24 h later by estimating hyperglycaemia and glycosuria (Visidex II and Diastix; Ames, Slough, UK). Plasma glucose samples were taken the day of final experiments.

Non-diabetic and newly induced diabetic groups were untreated or given a supplement of L-carnitine (Sigma, Poole, UK) in the drinking water for 1 month such that the dose was approximately 500 mg kg^{-1} day^{-1} based on a previous study using acetyl-L-carnitine (Cotter et al., 1995a). A further L-carnitine treated group was co-treated with the nitric oxide synthase inhibitor, N^G-nitro-L-arginine

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in the drinking water, at a dose of 10 mg kg^{-1} day^{-1}, which only causes modest conduction velocity changes in non-diabetic rats but blocks the effects of aldose reductase inhibitors in diabetic rats (Cameron et al., 1993, 1996). The duration of diabetes was chosen to give stable deficits in nerve conduction velocity and blood flow which do not change significantly over a 4-month period in this mature rat model, as recently reviewed (Cameron and Cotter, 1996a).

Rats were anaesthetised with thiobutabarbital sodium (Zeneca Pharmaceuticals, Macclesfield, UK) by intraperitoneal injection (50–100 mg kg^{-1}). The trachea was cannulated for artificial respiration and a carotid cannula was used to monitor blood pressure. Body core and nerve temperatures were kept in the range 37–38°C. Motor conduction velocity to tibialis anterior muscle was measured between sciatic notch and knee as previously described (Cameron et al., 1991a). Rats were then 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. Sciatic endoneurial blood flow was measured in the contralateral leg using microelectrode polarography and hydrogen clearance as previously described (Cameron et al., 1991b, 1996).

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 (composite blood flow and vascular 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. Where data were not normally distributed (fast blood flow component and conductance), they were subjected to Kruskal-Wallis non-parametric analysis of variance followed by Dunn's multiple comparison test and are expressed as median (lower-upper 95% confidence interval).

3. Results

Diabetic rats had a 5.6-fold increase in plasma glucose (Table 1) and lost approximately 25% of body weight over the 1-month period. These parameters were not significantly altered by L-carnitine or N^G-nitro-L-arginine treatments.

Sciatic motor conduction velocity (Fig. 1) was 19.8 ± 1.0% reduced ($P < 0.001$) after 1 month of diabetes. This was partially (54.7 ± 5.1%, $P < 0.001$) prevented by L-carnitine treatment, although conduction velocity remained 9.0 ± 1.0% ($P < 0.001$) slower than for the non-diabetic group. Joint treatment with N^G-nitro-L-arginine completely prevented ($P < 0.001$) the beneficial action of L-carnitine against the diabetic conduction deficit.

The 47.7 ± 4.2% reduction ($P < 0.001$) in endoneurial nutritive blood flow (Fig. 2A) after 1 month of untreated diabetes was attenuated to the extent of 48.6 ± 9.8% ($P < 0.001$) by L-carnitine treatment. N^G-Nitro-L-arginine co-treatment abolished the effect of L-carnitine ($P < 0.001$) on blood flow, the resultant value being in the untreated diabetic range. Systemic blood pressure (Fig. 2B) tended to be reduced in untreated (15.8 ± 3.0%, $P < 0.01$) or L-carnitine treated diabetic (21.8 ± 3.1%, $P < 0.001$) groups compared to non-diabetic controls. N^G-Nitro-L-arginine co-treatment increased pressure ($P < 0.05$) to a value not significantly different from the non-diabetic group. These perfusion pressure changes are taken into account by expressing the data as nutritive vascular conductance (Fig. 2C). The 38.3 ± 5.1% reduction ($P < 0.001$) in conductance with untreated diabetes was largely prevented by L-carnitine treatment ($P < 0.001$); the resultant value was not significantly different from that of the non-diabetic group. N^G-Nitro-L-arginine co-treatment completely prevented the effects of L-carnitine, depressing conductance 28.0 ± 5.6% below ($P < 0.05$) the untreated diabetic level.

Composite sciatic endoneurial blood flow and conductance (Table 2), the weighted sum of nutritive (capillary) and non-nutritive (arteriovenous shunt and large vessel) components recorded by hydrogen clearance, were decreased by 50.8 ± 5.0% ($P < 0.001$) and 40.4 ± 6.1% ($P < 0.01$) respectively after 1 month of diabetes. L-Carnitine

Table 1
Body weights and plasma glucose concentrations

Group	*n*	Body weight		Plasma glucose (mM)
		Start (g)	End (g)	
Control	12	449 ± 7		7.1 ± 0.4
Diabetic	12	466 ± 5	341 ± 9	40.6 ± 1.7
+ L-carnitine	12	459 ± 4	352 ± 8	41.3 ± 1.6
+ L-carnitine + N^G-nitro-L-arginine	10	466 ± 7	350 ± 12	38.6 ± 1.5

Data are mean ± S.E.M.

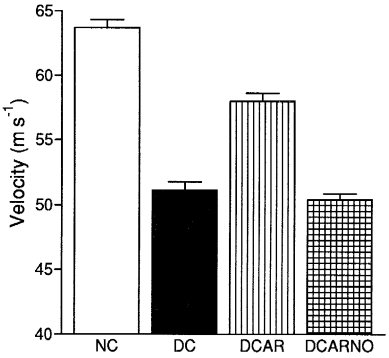

Fig. 1. Sciatic nerve motor conduction velocity values for fibres supplying tibialis anterior muscle. NC, non-diabetic control group; DC, 1-month diabetic control group; DCAR, 1-month diabetic group treated with L-carnitine (500 mg kg^{-1} day^{-1}) from induction; DCARNO, 1-month diabetic group treated with both L-carnitine and N^G-nitro-L-arginine (10 mg kg^{-1} day^{-1}) from induction. Group $n = 10$–12. Error bars are S.E.M.

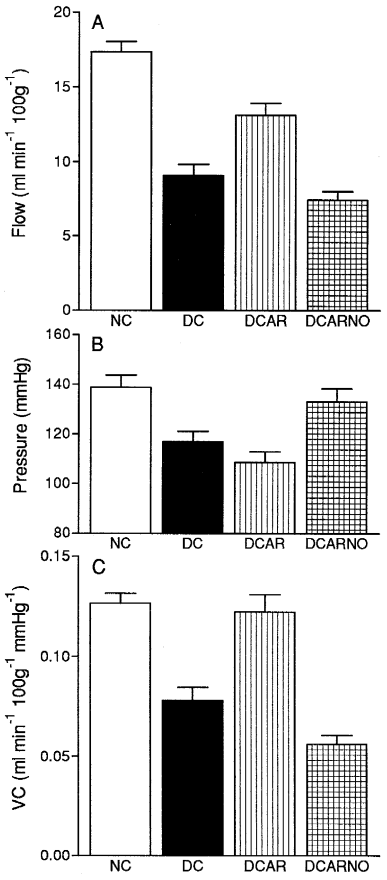

Fig. 2. Sciatic nerve nutritive perfusion parameters; (A) endoneurial nutritive blood flow, (B) mean systemic blood pressure, and (C) nutritive vascular conductance (VC). NC, non-diabetic control group; DC, 1-month diabetic control group; DCAR, 1-month diabetic group treated with L-carnitine (500 mg kg^{-1} day^{-1}) from induction; DCARNO, 1-month diabetic group treated with both L-carnitine and N^G-nitro-L-arginine (10 mg kg^{-1} day^{-1}) from induction. Group $n = 10$–12. Error bars are S.E.M.

treatment did not significantly alter composite flow or conductance in diabetic rats. In the N^G-nitro-L-arginine co-treatment group, composite flow was further depressed by approximately 61% compared to untreated ($P < 0.001$) or L-carnitine treated ($P < 0.001$) diabetic groups. A similar trend was seen in the composite conductance data. Compared to the non-diabetic group, the relative proportion of hydrogen clearance carried by the nutritive flow component tended to be slightly reduced by diabetes and restored by L-carnitine treatment, although neither of these trends was statistically significant compared to the non-diabetic group. However, for N^G-nitro-L-arginine co-treatment, the fast non-nutritive clearance component was only detected in 5/10 rats (compared to 12/12 for the other groups). Thus, the percentage of nutritive clearance tended to be elevated in this group, which was significant ($P < 0.01$) compared to untreated diabetes. The fast clearance tended to be reduced in diabetic and L-carnitine treated diabetic groups compared to non-diabetic rats; however, the data were very variable and this was not statistically significant. Co-treatment with N^G-nitro-L-arginine produced a marked further decrease in this component which was significantly depressed compared to the non-diabetic group for flow ($P < 0.001$) and compared to the diabetic and L-carnitine treated diabetic groups ($P < 0.05$) when the data were expressed as conductance.

Table 2
Non-nutritive endoneurial blood flow parameters

Group	n	Composite flow (ml min^{-1} 100 g^{-1})	Composite conductance (ml min^{-1} 100 g^{-1} mmHg^{-1})	Relative nutritive clearance (%)	Fast flow (ml min^{-1} 100 g^{-1})	Fast conductance (ml min^{-1} 100 g^{-1} mmHg^{-1})
Control	12	53.1 ± 5.5	0.380 ± 0.035	56.5 ± 4.5	96.6 (67.9–125.1)	0.743 (0.514–0.861)
Diabetic	12	26.2 ± 2.7 [a]	0.226 ± 0.023 [b]	41.2 ± 5.3	38.3 (29.6–52.8)	0.352 (0.267–0.452)
+ L-carnitine	12	27.2 ± 3.1 [a]	0.248 ± 0.092 [b]	59.4 ± 6.8	41.7 (25.6–57.8)	0.391 (0.238–0.501)
+ L-carnitine + N^G-nitro-L-arginine	10	10.4 ± 1.4 [a,c,f]	0.079 ± 0.034 [a,c,f]	77.4 ± 8.5 [d]	3.4 (1.2–21.2) [a]	0.020 (0.008–0.162) [a,e,g]

Data are mean ± S.E.M. or median (lower-upper 95% confidence interval). Statistics, [a] $P < 0.001$, [b] $P < 0.01$ versus non-diabetic group; [c] $P < 0.001$, [d] $P < 0.01$, [e] $P < 0.05$ versus diabetic group; [f] $P < 0.001$, [g] $P < 0.05$ versus diabetic + L-carnitine group.

4. Discussion

The data show that L-carnitine treatment partially prevented the development of nerve conduction deficits in diabetic rats to an extent comparable to that observed with similar doses of acetyl- and proprionyl-L-carnitine (Cotter et al., 1995a). This finding is also in agreement with another study where L-carnitine (about 1.5 times the dose used in this study) prevented a defect in caudal motor conduction velocity (Ido et al., 1994).

L-Carnitine treatment elevated endoneurial nutritive blood flow in diabetic rats, roughly in proportion to the effects on conduction velocity. This is in agreement with observations for acetyl- and proprionyl-L-carnitine (Cotter et al., 1995a). However, the change in blood flow was largely restricted to the nutritive component, total endoneurial flow remaining at the diabetic control level. This suggests that treatment effects were specific to the vascular elements controlling perfusion of the endoneurial capillary bed, rather than having a generalised action to also increase arteriovenous shunt flow. Further support to this notion is given by the lack of effect of L-carnitine treatment on the fast clearance component. While several pharmacological manipulations have a widespread action on vasa nervorum, for example $n-6$ essential fatty acids, nitrovasodilators and adrenoceptor antagonists (reviewed in Cameron and Cotter, 1994) some other treatments have a similar specific effect, including aldose reductase inhibitors and aminoguanidine (Cameron and Cotter, 1996b; Cameron et al., 1994b, 1996).

The results for nerve perfusion using the hydrogen clearance method are at variance with an interpretation of data obtained using a microsphere capture technique (Ido et al., 1994). Under those conditions diabetes caused greater entrapment of microspheres in the vessels of several tissues including sciatic nerve, which was taken to suggest an increase in blood flow. L-Carnitine and acetyl-L-carnitine had no effect on microsphere capture by nerve vessels, hence it was considered that they did not have a vascular action. However, a previous study in granulation tissue did show a reduction in microsphere entrapment by acetyl-L-carnitine, leading to the opposite conclusion (Williamson and Arrigoni-Martelli, 1992).

Evidence from hydrogen clearance measurements that L-carnitine has a neurovascular action in diabetes is supported by the results for combined treatment with N^G-nitro-L-arginine. Thus, partial blockade of nitric oxide synthase, which is primarily localised in endothelium and nitrergic innervation of epi- and peri-neurial vessels (Yagihashi, 1995), abolished the protective effect of L-carnitine on both blood flow and conduction velocity. A similar antagonism was noted when nitric oxide synthase inhibition was combined with aldose reductase inhibitors, antioxidants and aminoguanidine (Cameron and Cotter, 1995, 1996b; Cameron et al., 1996; Stevens et al., 1994); the latter 3 treatments normally increase nerve blood flow and conduction velocity and improve nitric oxide mediated endothelium-dependent vasorelaxation in diabetic rats (Archibald et al., 1996; Cameron and Cotter, 1992; Keegan et al., 1995). In contrast, nitric oxide synthase inhibition did not alter conduction velocity responses to myo-inositol, which does not affect nerve blood flow and presumably acts 'downstream' of the vasculature, directly on nerve fibres (Cameron et al., 1994b; Stevens et al., 1994). The precise nature of the vascular effect of L-carnitine and its derivatives is unknown. Although blocked by nitric oxide synthase inhibition, this is not sufficient evidence to establish a mode of action via the nitric oxide system (Cameron et al., 1996). Thus, L-carnitine could have a direct vasodilator effect on vascular smooth muscle, which would be indirectly opposed when endothelium dependent relaxation was reduced by nitric oxide synthase blockade.

A further potential mechanism for the vascular action of L-carnitine derivatives is as an antioxidant, which has been noted in vivo (Fariello and Calabrese, 1988) and in vitro (Tesco et al., 1992). Both lipophilic and hydrophilic free radical scavengers protect against the development of blood flow and conduction abnormalities in diabetic rats (Bravenboer et al., 1992; Cameron et al., 1994a). The high dose of L-carnitine used in these experiments is compatible with the high doses of free radical scavengers necessary to protect nerve function (Cotter et al., 1995b). Interestingly, acetyl-L-carnitine treatment prevented the accumulation of a marker of lipid peroxidation in sciatic nerve of diabetic rats (Lowitt et al., 1995).

Scavengers such as α-tocopherol also reduce some metabolic consequences of diabetes or hyperglycaemia by preventing an increase in membrane diacylglycerol in rat aorta and smooth muscle cells (Kunisaki et al., 1994). Elevated diacylglycerol stimulates protein kinase C, which in turn has been linked to reduced endothelium-dependent relaxation (Cohen, 1993; Mayhan and Patel, 1995). Treatment of diabetic rats with α-tocopherol prevents the development of impairments in endothelium-dependent relaxation, nerve blood flow, and conduction velocity (Cotter et al., 1995b; Keegan et al., 1995). In diabetic nerve, diacylglycerol levels are reduced in contrast to aorta where they are increased (Ido et al., 1994). The consequent reduction in protein kinase C activation has been suggested to be responsible for a reduction in nerve Na^+,K^+-ATPase activity. In turn this has been hypothesised to cause conduction velocity deficits (Stevens et al., 1994). However, vasodilator studies show that normal conduction velocity can be found in the presence of a diabetic level of Na^+,K^+-ATPase activity (Cameron et al., 1991a) suggesting that this biochemical deficit is not severe enough to impair nerve function. Treatment with L-carnitine derivatives caused a further reduction in diabetic nerve diacylglycerol content (Ido et al., 1994) yet conduction velocity increased. This argues against a direct link between diacylglycerol/protein kinase C mechanisms and nerve function. However, if L-carnitine also reduces vasa nervorum diacylglycerol then

there would be an indirect link due to vasodilation because of reduced activation of protein kinase C, perhaps via increased nitric oxide production as noted for aorta (Cohen, 1993; Kunisaki et al., 1994). This hypothesis explains the effects of L-carnitine on nerve blood flow and conduction velocity, and their opposition by N^G-nitro-L-arginine. Protein kinase C inhibitors also attenuate vasoconstrictor-induced increase in vascular smooth muscle tone in vitro (Shimamoto et al., 1993). Thus, reduced vessel diacylglycerol could account for the neurovascular effects of L-carnitine, in line with expectations from the increased long chain fatty acids transport into mitochondria for β-oxidation (Stevens et al., 1995).

In conclusion, L-carnitine treatment partially protects nerve function against the effects of diabetes, primarily by an action on vasa nervorum to increase nutritive endoneurial perfusion. This could prove useful in the treatment of neuropathy in patients and the efficacy of L-carnitine derivatives is currently being assessed in clinical trials.

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