ELSEVIER

European Journal of Pharmacology 314 (1996) 33–39

ejp

Nerve function and regeneration in diabetic and galactosaemic rats: antioxidant and metal chelator effects

Alastair Love, Mary A. Cotter, Norman E. Cameron *

Department of Biomedical Sciences, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland, UK

Received 5 February 1996; revised 23 May 1996; accepted 28 June 1996

Abstract

Immature rats were made diabetic with streptozotocin or were fed a 40% galactose diet to stimulate the polyol pathway. Separate diabetic and galactosaemic groups were treated with butylated hydroxytoluene or trientine. After 4 weeks the sciatic nerve was freeze-lesioned. Two weeks later, the degree of myelinated fibre regeneration was assessed electrophysiologically and nerve conduction velocity was measured in the contralateral leg. Similar sciatic motor and saphenous sensory nerve conduction velocity deficits of approximately 18% and 19%, respectively, compared to age-matched control rats were found in both models. They were partially prevented by treatment (approximately 68% for butylated hydroxytoluene and 63% for trientine). There were 12% and 10% deficits in nerve regeneration distance with diabetes and galactosaemia respectively, which were markedly attenuated (approximately 80%) by both treatments. The data emphasise the importance of elevated free radical activity for the aetiology of neural/neurovascular deficits in experimental diabetes and galactosaemia.

Keywords: Polyol pathway; Diabetes; Galactosemia; Nerve conduction; Nerve regeneration; Oxygen free radical; Antioxidant; Metal chelator

1. Introduction

The early abnormalities in peripheral nerve function in experimental diabetes mellitus, such as reduced nerve conduction velocity, are largely caused by impaired endoneurial blood flow that results in a hypoxic microenvironment for axons and Schwann cells (Cameron and Cotter, 1994; Low et al., 1989). Similar changes are seen in neuropathic diabetic patients (Tesfaye et al., 1994). Decreased vasa nervorum perfusion has been linked with elevated polyol pathway flux and increased oxygen free radical activity in diabetic rats (Cameron et al., 1994; Calcutt et al., 1994b; Cotter et al., 1995; Nagamatsu et al., 1995) as well as increased formation of advanced glycosylation end products (Kihara et al., 1991) and reduced ω-6 essential fatty acid metabolism (Cameron and Cotter, 1994). The polyol pathway is activated by hyperglycaemia which stimulates production of sorbitol from glucose by aldose reductase (L-alditol:$NADP^+$ 1-oxidoreductase; EC 1.1.1.21). Sorbitol is then further metabolized to fructose by the second enzyme in the pathway, sorbitol dehydrogenase (L-iditol:NAD^+ 5-oxidoreductase; EC 1.1.1.14) (Dvornik, 1987). Excessive flux through the first half of the polyol pathway may impair free radical scavenging by interfering with the glutathione redox cycle (Kashiwagi et al., 1994). Other explanation of polyol effects, stressing the importance of the second half of the pathway, have also been proposed. These include involvement of fructose in the advanced glycosylation process (Brownlee, 1992) and NADH accumulation causing deleterious alterations to intermediate metabolism (Williamson et al., 1993). In another model of polyol pathway activation, the galactosaemic rat, excess flux is restricted to the first half of the pathway, as the sugar alcohol galactitol is a very poor substrate for sorbitol dehydrogenase (Dvornik, 1987). Nevertheless, conduction velocity reductions are similar to those of diabetic rats (Cameron et al., 1992; Low et al., 1989). One aim of the investigation was to determine whether conduction velocity deficits in galactosaemic rats could be prevented by treatments which either scavenge oxygen free radicals (Cameron et al., 1993) or inhibit their formation by the processes of autoxidation and advanced glycosylation which are catalysed by small amounts of free transition metal ions, using an appropriate chelating agent (Cameron and Cotter, 1995).

* Corresponding author. Tel.: (44-1224) 273-013; Fax: (44-1224) 273-019.

PII S0014-2999(96)00528-6

Experimental diabetes and galactosaemia are characterised by defects in nerve regeneration (Longo et al., 1986; Powell et al., 1986). The extent to which these changes depend on similar mechanisms to those responsible for reduced conduction velocity, as opposed to being caused by impaired metabolic or neurotrophic responses (Vinik et al., 1995) is not known. Therefore a second aim was to examine the effects of antioxidant and transition metal chelator treatments on regeneration of myelinated fibres following freeze damage in diabetic and galactosaemic rats.

2. Materials and methods

All experiments were carried out on male Sprague-Dawley rats (Aberdeen University Colony), 8 weeks old at the start of the study. Diabetes was induced with intraperitoneal streptozotocin (Zeneca Pharmaceuticals, Macclesfield, Cheshire, UK) freshly dissolved in sterile saline at a dose of 55 mg kg^{-1}. Galactosaemia was produced by feeding rats a 40% galactose diet (Cameron et al., 1992). Separate groups of diabetic and galactosaemic rats were treated from the day after induction with a dietary supplement of the lipophilic antioxidant, butylated hydroxytoluene (Sigma, Poole, Dorset, UK), such that the dose was approximately 1 g kg^{-1} day^{-1}, based on measurements of daily food consumption of 40–50 g by diabetic and galactosaemic rats. Further diabetic and galactosaemic groups were treated from the second day with the transition metal chelator, trientine (triethylenetetramine dihydrochloride, Sigma), dissolved in the drinking water such that the dose was approximately 20 mg kg^{-1} day^{-1}. The drinking water concentration for diabetic and galactosaemic rats was adjusted to compensate for their different water consumption, 250–300 ml day^{-1} for the former, 40–45 ml day^{-1} for the latter. Pilot dose-ranging investigations (Cameron and Cotter, unpublished observations) previously established that the drug treatments used were near the top of their respective dose-response curves for correction of motor conduction velocity in mature diabetic rats and this was confirmed in later studies (Cameron and Cotter, 1995; Cameron et al., 1993).

After 4 weeks, diabetic, galactosaemic and age-matched control rats were anaesthetized with halothane (2–5% in air), and under aseptic conditions, the right sciatic nerve was exposed in the upper thigh region. The nerve was lesioned using a liquid N_2 cooled probe, 1 mm in diameter, which was lightly applied to the nerve for 50 s. This produced a punctate lesion that caused all fibres in the nerve to degenerate (Maxfield et al., 1995). The lesion site was marked by an epineurial suture. Rats were then left a further 14 days before regeneration was assessed.

In final experiments, rats were anaesthetized with urethane (1–1.5 g kg^{-1} i.p.). Motor and sensory conduction velocity were measured in the left (non-lesioned) legs of the rats using methods previously described in detail (Cameron et al., 1992). A group of onset (8 week old) control rats was also used for conduction velocity studies. Briefly, sciatic motor conduction velocity between sciatic notch and knee was taken as the average value for branches supplying gastrocnemius and tibialis anterior muscles. Saphenous sensory conduction was estimated between groin and lower calf. Core and nerve temperatures were monitored by thermocouple probes and kept in the range 37–38°C by radiant heat.

The electrophysiological method used to estimate regeneration distance was similar to that described by Bondoux-Jahan and Sebille (1989). The right (lesioned) sciatic/tibial nerve was dissected free between sciatic notch and ankle and placed in a nerve bath on an array of electrodes (1 mm spacing) at the in vivo length. Bath temperature was 35°C, and the nerve/electrode complex was suspended above Krebs' solution, gassed with 95% O_2/5% CO_2. Thus, nerves were maintained in a warm, moist, oxygenated environment where they remained viable without significant deterioration for at least 4 h. Nerve stimulation parameters were adjusted to be supramaximal for myelinated fibres (1 Hz, 10 mA, pulse width < 100 s), assessed by monitoring the size of the first peak of the compound action potential near the lesion site. Stimulation was applied 3–8 mm proximal to the lesion. Small myelinated (Aδ) and unmyelinated (C) fibres were not activated with these stimulation parameters. Bipolar stimulation was used to minimise the stimulus artefact. Compound action potential recordings were made along the length of the nerve at 1 mm intervals using a bipolar electrode configuration and signal averaging. The point at which the compound action potential was no longer detectable was taken to indicate the maximum regeneration distance.

2.1. Statistical analysis

Data are expressed as the group means ± S.E.M. They were subjected to Bartlett's test for homogeneity of variances followed by a one-way analysis of variance. When significance was attained ($P < 0.05$) between-group differences were established using the Student-Newman-Keuls multiple comparison test. All calculations were performed using standard statistical software (Instat, Graphpad, San Diego, CA, USA).

3. Results

Body weights (Table 1) increased with age by approximately 38% ($P < 0.001$) in control rats. With diabetes, there was an approximately 25% weight loss ($P < 0.01$), whereas with galactosaemia there was a reduced growth rate (19%) compared to controls. The latter was unaffected by trientine treatment but rats in the butylated hydroxytoluene-treated galactosaemic group had a lower weight

Table 1
Body weights and plasma glucose concentrations

Group	n	Body weight (g)		Plasma glucose concentration (mM)
		Start	End	
Onset control	8	276 ± 6		8.1 ± 0.5
Age-matched control	15		382 ± 5	9.0 ± 0.4
Diabetic	15	261 ± 4	218 ± 9	42.9 ± 2.0
Diabetic + butylated hydroxytoluene	14	285 ± 6	196 ± 6	42.6 ± 2.0
Diabetic + trientine	11	284 ± 5	204 ± 10	48.4 ± 2.7
Galactosaemic	16	267 ± 6	317 ± 5	8.8 ± 0.3
Galactosaemic + butylated hydroxytoluene	15	278 ± 5	247 ± 13	8.2 ± 0.3
Galactosaemic + trientine	11	272 ± 4	311 ± 5	8.2 ± 0.4

Data are the means ± S.E.M.

than either untreated or trientine-treated galactosaemic rats ($P < 0.01$). Nerve water content was 68.7 ± 1.4% in control rats and this was not significantly affected by diabetes (70.4 ± 0.8%). With galactosaemia, water content was significantly ($P < 0.01$) elevated compared to controls being 78.5 ± 1.5%, 76.6 ± 0.4% and 76.7 ± 1.5% for untreated, butylated hydroxytoluene- and trientine-treated groups, respectively.

Sciatic motor conduction velocity (Fig. 1) increased in control rats by 30.0 ± 1.8% ($P < 0.001$) over the 6-week experimental period. With diabetes, maturation of motor conduction velocity was restricted to 11.1 ± 1.4%, which, although significantly elevated compared to the onset control group ($P < 0.001$), was reduced compared to the age-matched group ($P < 0.001$). Butylated hydroxytoluene and trientine treatments improved the conduction deficit by 74.6 ± 6.5% ($P < 0.001$) and 55.8 ± 6.5% ($P < 0.001$), respectively, although for both butylated hydroxytoluene ($P < 0.05$) and trientine ($P < 0.01$) there remained small but statistically significant differences compared to the age-matched control group. Galactosaemia blunted motor conduction velocity development to an extent similar to diabetes, with a 13.8 ± 1.1% ($P < 0.001$) increase compared to onset controls, which fell short of the value for the age-matched group ($P < 0.001$). Treatment with butylated hydroxytoluene and trientine largely prevented the conduction deficit to the extent of 62.0 ± 8.3% ($P < 0.001$) and 69.8 ± 11.8% ($P < 0.001$), respectively. For butylated hydroxytoluene, the remaining conduction deficit just reached statistical significance ($P < 0.05$) compared to age-matched controls.

Saphenous sensory conduction velocity (Fig. 2) showed an age-related increase of 21.7 ± 1.1% ($P < 0.001$) in control rats. This was almost completely prevented by diabetes (2.9 ± 1.0% increase, $P > 0.05$) and galac-

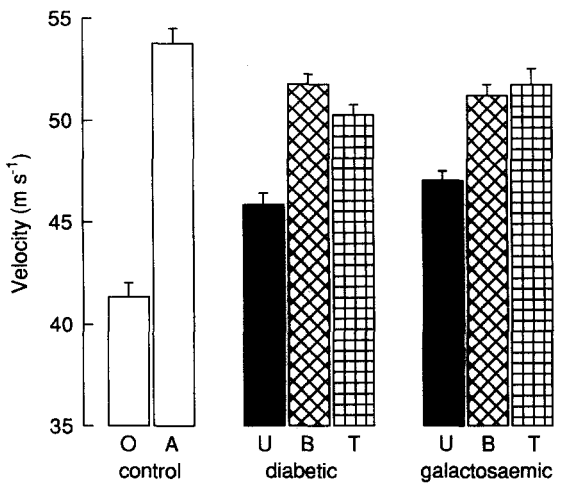

Fig. 1. Average sciatic nerve motor conduction velocity values for fibres supplying tibialis anterior and gastrocnemius muscles. For control groups O, onset control, A, age-matched control; diabetic and galactosaemic groups U, untreated, B, treated with butylated hydroxytoluene (1 g kg^{-1} day^{-1}), T, treated with trientine (20 mg kg^{-1} day^{-1}). Group $n = 8$–16, error bars are S.E.M.

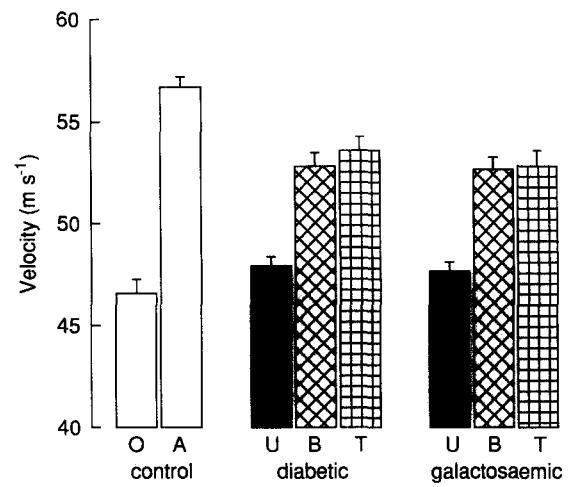

Fig. 2. Saphenous nerve sensory conduction velocity. For control groups, O, onset control, A, age-matched control; diabetic and galactosaemic groups, U, untreated, B, treated with butylated hydroxytoluene (1 g kg^{-1} day^{-1}), T, treated with trientine (20 mg kg^{-1} day^{-1}). Group $n = 8$–16, error bars are S.E.M.

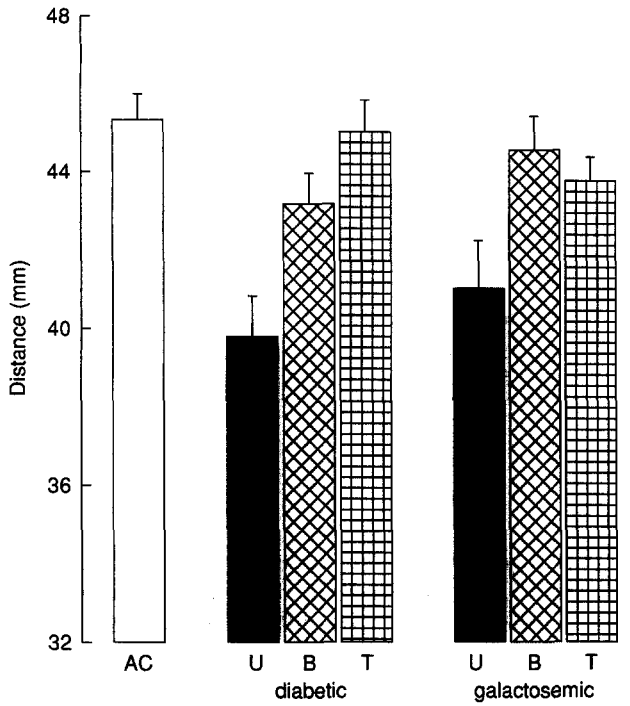

Fig. 3. Maximum myelinated fibre regeneration distance 14 days after a sciatic nerve lesion. For age-matched control (AC), diabetic and galactosaemic groups U, untreated, B, treated with butylated hydroxytoluene (1 g kg^{-1} day^{-1}) and T, treated with trientine (20 mg kg^{-1} day^{-1}). Group $n = 9–15$, error bars are S.E.M.

tosaemia ($2.4 \pm 0.9\%$ increase, $P > 0.05$), the conduction velocity values remaining significantly ($P < 0.001$) below that of the age-matched control group. Treatment with butylated hydroxytoluene or trientine increased sensory conduction velocity in diabetic and galactosaemic rats to a similar extent. Thus, the diabetic deficit was prevented by $55.7 \pm 7.5\%$ ($P < 0.001$) for butylated hydroxytoluene and $64.7 \pm 8.0\%$ ($P < 0.001$) for trientine. Corresponding values for galactosaemia were $55.3 \pm 6.7\%$ ($P < 0.001$) and $57.2 \pm 8.5\%$ ($P < 0.001$). However, for all treated rats, there remained a significant ($P < 0.001$) deficit compared to the age-matched control group. There were no instances where diabetic or treated diabetic motor and sensory conduction velocity values were significantly different from their galactosaemic counterparts.

The maximum regeneration distance for myelinated sciatic nerve fibres 14 days after a punctate freeze lesion (Fig. 3) was $12.3 \pm 2.3\%$ reduced by diabetes ($P < 0.01$). This was completely prevented by trientine treatment ($P < 0.01$) and butylated hydroxytoluene attenuated the deficit by $60.9 \pm 14.2\%$ ($P < 0.05$), the resultant value being in the lower half of the control range. Galactosaemia caused a $9.6 \pm 2.7\%$ deficit ($P < 0.05$), which was completely prevented by butylated hydroxytoluene ($P < 0.05$) and $63.0 \pm 14.3\%$ reduced by trientine, although in the latter case this did not reach statistical significance compared to untreated galactosaemia.

4. Discussion

The data emphasise the similarity of nerve conduction velocity and regeneration deficits in young diabetic and galactosaemic rats. Butylated hydroxytoluene, a lipophilic chain-breaking scavenger, is likely to distribute widely in cell membranes and circulating lipids and should cross the blood-nerve barrier (Cameron et al., 1993; Low et al., 1989). Trientine is a hydrophilic metal chelator and can therefore reduce free radical formation by the Fenton reaction, autoxidation, and advanced glycosylation, which are catalysed by free transition metal ions (Fu et al., 1994). It is not known whether trientine crosses the blood-nerve barrier. In diabetic rats, both lipophilic and hydrophilic scavengers and transition metal chelators prevent or correct impaired nerve blood flow and conduction (Bravenboer et al., 1992; Cameron and Cotter, 1995; Cameron et al., 1993; Cotter et al., 1995; Nagamatsu et al., 1995). This study extends the conduction effect to the galactosaemic model. Many studies have used mature diabetic rats, where a conduction velocity deficit relative to onset controls is found (Cameron and Cotter, 1995; Cameron et al., 1993; Cotter et al., 1995). In this model there is a minimal contribution of impaired nerve fibre growth to reduced conduction velocity, the pathophysiological deficits relating primarily to neuronal adaptations to endoneurial hypoxia (reviewed in Cameron and Cotter, 1994). In contrast, for the young rats used in this study, there is substantial blunting of axon growth which has been suggested to make a major contribution to impaired conduction velocity (Cameron et al., 1986; Sharma et al., 1981; Yagihashi, 1995). However, prevention by butylated hydroxytoluene and trientine treatments, suggests that similar mechanisms underlie conduction deficits in young and mature models, irrespective of whether or not they are caused by reduced axon growth. The data agree with a previous study in younger rats, where diabetic motor and sensory conduction deficits were partially prevented by GSH treatment (Bravenboer et al., 1992).

Part of the reason for the similarity of motor and sensory conduction reductions in young rats with diabetes or galactosaemia may stem from the negative effects of both on axonal transport of cytoskeletal components and their assembly into neurofilaments – a major determinant of axon calibre, hence conduction velocity (Nukuda et al., 1986; Yagihashi, 1995). The body weight decrease in diabetic or the blunted increase in galactosaemic rats would not be expected to contribute to reduced conduction velocity. Such a hypothesis cannot explain the beneficial conduction effects of butylated hydroxytoluene and trientine treatments, which did not improve body weight. The butylated hydroxytoluene-treated galactosaemic rats actually lost weight compared to untreated galactosaemia, yet their conduction velocity was markedly increased. The reason for the weight loss is unclear; it was not accompanied by a reduced food intake. In normal rats, a dietary caloric

restriction severely reduced weight but had no effect on conduction velocity maturation (Cornblath and Brown, 1988). The reduced blood flow and endoneurial hypoxia characteristic of diabetes (Cameron and Cotter, 1994; Low et al., 1989) and the decreased perfusion and oedema-dependent increase in oxygen diffusion distance in galactosaemia (McManis et al., 1986; Myers and Powell, 1984) could limit energy-dependent neuronal processes, including neurofilament synthesis, transport and assembly. This would impair conduction velocity maturation. In mature diabetic and galactosaemic rats, vasodilator treatment improves conduction velocity (Cameron and Cotter, 1994; Dines et al., 1995), and this is also the case in younger diabetic rats (Kapelle et al., 1992).

The main parallel between diabetes and galactosaemia is increased activation of the first half of the polyol pathway to produce sorbitol or galactitol (Dvornik, 1987). Thus, the similarity of nerve conduction and regeneration deficits in the 2 models emphasizes the importance of this step, as opposed to changes linked to the conversion of sorbitol to fructose by sorbitol dehydrogenase (Williamson et al., 1993) which is specific to diabetes. Antioxidant treatment does not alter nerve polyol pathway activity (Cameron et al., 1993) and butylated hydroxytoluene and trientine did not affect the increase in nerve water content in galactosaemia. However, exaggerated flux through the aldose reductase step of the polyol pathway can deplete cellular NADPH (Dvornik, 1987). For vascular endothelial cells this increases the susceptibility to peroxide damage because of an impaired glutathione redox cycle (Kashiwagi et al., 1994). Reduced protection against reactive oxygen species, coupled with their increased production by autoxidative glycosylation (Cameron and Cotter, 1995), causes defective endothelium-dependent vessel relaxation in diabetes and galactosaemia. This is prevented by aldose reductase inhibitors, scavengers including butylated hydroxytoluene, and trientine (Archibald et al., 1996; Cameron and Cotter, 1993, 1994; Keegan et al., 1996). Vasa nervorum endothelial dysfunction is likely to make a major contribution to reduced nerve conduction (Cameron and Cotter, 1994; Low et al., 1989) in diabetes and galactosaemia.

The data on myelinated fibre regeneration in diabetic and galactosaemic rats agrees with previous studies showing similar defects in growth through a silicon tube following nerve transection (Longo et al., 1986; Powell et al., 1986). In contrast, an experiment monitoring regeneration by the pinch test did not find an impairment in galactosaemic rats 7 days after nerve crush (Calcutt et al., 1994a). However, 7 days may be too short for a statistically viable deficit to develop in galactosaemic rats, although the pinch test can detect reduced regeneration over this time in diabetic rats (Ekström and Tomlinson, 1989). The pinch test depends on evoking flexor reflexes in proximal muscles, thus monitoring regeneration in small sensory fibres involved in nociception. In contrast, aldose reductase is localised to Schwann cell cytoplasm of the larger myelinated fibres examined in this study, rather than small fibres (Powell et al., 1991). While conduction velocity and regenerative capacity of myelinated fibres are similarly affected by polyol pathway-dependent mechanisms in diabetes and galactosaemia, there may be differences in small fibre regeneration due to other factors (Calcutt et al., 1994a; Ekström and Tomlinson, 1989). Thus, nerve growth factor is reduced in diabetic rats and replacement therapy corrects small sensory but not large fibre function (Apfel et al., 1994). It is not known whether galactosaemic rats have a nerve growth factor deficit.

In diabetic and particularly galactosaemic rats there is a marked reduction in nerve ciliary neurotrophic factor content, probably due to Schwann cell damage caused by polyol pathway activation (Calcutt et al., 1992). This may contribute to reduced regenerative responses and conduction velocity maturation both of which are corrected by ciliary neurotrophic factor treatment in young diabetic rats (Cameron et al., 1995). However, the precise mechanism whereby diabetes and galactosaemia influence neurotrophin levels is not known. Partial prevention of defective regeneration by butylated hydroxytoluene and trientine suggests two possibilities. First, Schwann cell disruption may not result simply from the osmotic shock of polyol accumulation. Instead, it could depend on increased susceptibility to free radical damage due to an impaired glutathione redox cycle as noted for endothelial cells (Kashiwagi et al., 1994). Nerve GSH levels are reduced and lipid peroxidation is increased with a diabetes duration similar to that of this study, protection being given by antioxidant treatment (Nagamatsu et al., 1995). However, the status of nerve antioxidant defences and the level of free radical lipid damage are not known in galactosaemia. Second, Schwann cell polyol pathway activity may be only partially relevant, the main damage resulting indirectly as from impaired nerve perfusion and ischaemia/reperfusion effects caused by endothelial dysfunction. In diabetic and galactosaemic nerve conduction defects are at least partially corrected by vasodilator treatments which do not alter polyol pathway metabolite concentrations (Cameron and Cotter, 1994; Dines et al., 1995; Maxfield et al., 1995). Moreover, in mature diabetic rats, the regeneration deficit 14 days post-lesion was completely prevented by vasodilator therapy that promoted normal endoneurial oxygen tension (Maxfield et al., 1995). In non-diabetic rats, nerve regeneration is accompanied by endoneurial angiogenesis, presumably to meet the increased metabolic needs of growing fibres (Nukuda, 1988). Furthermore, the early phase of regeneration through a nerve transplant was markedly accelerated if the graft had an intact vascular supply (Shupek et al., 1989). Thus, this stresses the importance of neurovascular insufficiency leading to an inadequate endoneurial microenvironment for regeneration as well as conduction in diabetes and galactosaemia.

In conclusion, the data emphasise the importance of free radical mechanisms in diabetic and galactosaemic

nerve dysfunction. They suggest that galactosaemia is a good model for aspects of diabetic neuropathy, particularly myelinated fibre growth and regeneration and microangiopathy.

Acknowledgements

This research was funded by the British Diabetic Association. N.E.C. was supported by a Wellcome Trust Research Leave Fellowship.

References

Apfel, S.C., J.C. Arezzo, M. Brownlee, H. Federoff and J.A. Kessler, 1994, Nerve growth factor administration protects against experimental diabetic sensory neuropathy, Brain Res. 634, 7.

Archibald, V., M.A. Cotter, A. Keegan and N.E. Cameron, 1996, Contraction and relaxation of aortas from diabetic rats: effects of chronic anti-oxidant and aminoguanidine treatments, Naunyn-Schmiedeberg's Arch. Pharmacol. 353, 584.

Bondoux-Jahan, M. and A. Sebille, 1989, Conditioning lesion effects on rat sciatic nerve regeneration are influenced by electrical stimulation delivered to denervated muscles, Brain Res. 490, 350.

Bravenboer, B., A.C. Kapelle, F.P.T. Hamers, T. Van Buren, D.W. Erkelens and W.H. Gispen, 1992, Potential use of glutathione for the prevention and treatment of diabetic neuropathy in the streptozotocin-induced diabetic rat, Diabetologia 35, 813.

Brownlee, M., 1992, Glycation products and the pathogenesis of diabetic complications, Diabetes Care 15, 1835.

Calcutt, N.A., D. Muir, H.C. Powell and A.P. Mizisin, 1992, Reduced ciliary neuronotrophic factor-like activity in nerves from diabetic or galactose-fed rats, Brain Res. 575, 320.

Calcutt, N.A., H.F. McMurray, D.F. Moorehouse, M. Bache, S. Parthasarathy, H.C. Powell and A.P. Mizisin, 1994a, Inhibition of macrophage chemotaxis and peripheral nerve regeneration in normal and hyperglycemic rats by the aldose reductase inhibitor, tolrestat, Exp. Neurol. 128, 262.

Calcutt, N.A., A.P. Mizisin and M.W. Kalichman, 1994b, Aldose reductase inhibition, Doppler flux and conduction in diabetic rat nerve, Eur. J. Pharmacol. 251, 27.

Cameron, N.E. and M.A. Cotter, 1993, Contraction and relaxation of aortas from galactosaemic rats and the effects of polyol pathway inhibition, Eur. J. Pharmacol. 243, 47.

Cameron, N.E. and M.A. Cotter, 1994, The relationship of vascular changes to metabolic factors in diabetes mellitus and their role in the development of peripheral nerve complications, Diabetes Metab. Rev. 10, 189.

Cameron, N.E. and M.A. Cotter, 1995, Neurovascular dysfunction in diabetic rats; potential contribution of autoxidation and free radicals examined using transition metal chelating agents, J. Clin. Invest. 96, 1159.

Cameron, N.E., M.B. Leonard, I. Ross and P. Whiting, 1986, The effects of sorbinil on peripheral nerve conduction velocity, polyol concentrations and morphology in the streptozotocin diabetic rat, Diabetologia 29, 168.

Cameron, N.E., M.A. Cotter, S. Robertson and D. Cox, 1992, Muscle and nerve dysfunction in rats with experimental galactosaemia, Exp. Physiol. 77, 89.

Cameron, N.E., M.A. Cotter and E.K. Maxfield, 1993, Antioxidant treatment prevents the development of peripheral nerve dysfunction in streptozotocin-diabetic rats, Diabetologia 36, 299.

Cameron, N.E., M.A. Cotter, K.C. Dines, E.K. Maxfield, F. Carey and D. Mirrlees, 1994, Aldose reductase inhibition, nerve perfusion, oxygenation and function in streptozotocin-diabetic rats: dose-response considerations and independence from a myo-inositol mechanism, Diabetologia 37, 651.

Cameron, N.E., A. Love and M.A. Cotter, 1995, Ciliary neuronotrophic factor improves nerve conduction and regeneration in experimental diabetes, Diabetologia 38 (Suppl. 1), A233.

Cornblath, D.R. and M.J. Brown, 1988, Influence of malnutrition on developing rat peripheral nerves, Exp. Neurol. 99, 403.

Cotter, M.A., A. Love, M.J. Watt, N.E. Cameron and K.C. Dines, 1995, Effects of natural free radical scavengers on peripheral nerve and neurovascular function in diabetic rats, Diabetologia 38, 1285.

Dines, K.C., M.A. Cotter and N.E. Cameron, 1995, Nerve function in galactosaemic rats: effects of evening primrose oil and doxazosin, Eur. J. Pharmacol. 281, 303.

Dvornik, D., 1987, Hyperglycemia in the pathogenesis of diabetic complications, in: Aldose Reductase Inhibition. An Approach to the Prevention of Diabetic Complications, ed. D. Porte (Biomedical Information Corporation, New York, NY) p. 69.

Ekström, P.A.R. and D.R. Tomlinson, 1989, Impaired nerve regeneration in streptozotocin-diabetic rats. Effects of treatment with an aldose reductase inhibitor, J. Neurol. Sci. 93, 231.

Fu, M.-X., K.J. Wells-Knecht, J.A. Blackledge, T.J. Lyons, S.R. Thorpe and J.W. Baynes, 1994, Glycation, glycoxidation and cross-linking of collagen by glucose: kinetics, mechanisms, and inhibition of late stages of the Maillard reaction, Diabetes 43, 676.

Kapelle, A.C., B. Bravenboer, J. Traber, D.W. Erkelens and W.H. Gispen, 1992, The Ca^{2+} antagonist nimodipine counteracts the onset of an experimental neuropathy in streptozotocin induced, diabetic rats, Neurosci. Res. Commun. 10, 95.

Kashiwagi, A., T. Asahina, M. Ikebuchi, Y. Tanaka, Y. Takagi, Y. Nishio, R. Kikkawa and Y. Shigeta, 1994, Abnormal glutathione metabolism and increased cytotoxicity caused by H_2O_2 in human umbilical vein endothelial cells cultured in high glucose medium, Diabetologia 37, 264.

Keegan, A., V. Archibald, M.A. Cotter and N.E. Cameron, 1996, Transition metal chelators and anti-oxidants prevent the development of defective endothelium-dependent relaxation in aortas from diabetic rats, Diabetic Med. 13 (Suppl. 1), P9.

Kihara, M., J.D. Schmelzer, J.F. Poduslo, F.F. Curran, K.K. Nickander and P.A. Low, 1991, Aminoguanidine effect on nerve blood flow, vascular permeability, electrophysiology, and oxygen free radicals, Proc. Natl. Acad. Sci. USA 88, 6107.

Longo, F.M., H.C. Powell, J. LeBeau, M.R. Gerrero, B.S. Heckman and R.R. Myers, 1986, Delayed nerve regeneration in streptozotocin diabetic rats, Muscle Nerve 9, 385.

Low, P.A., T.D. Lagerlund and P.G. McManis, 1989, Nerve blood flow and oxygen delivery in normal, diabetic and ischemic neuropathy, Int. Rev. Neurobiol. 31, 355.

Maxfield, E.K., A. Love, M.A. Cotter and N.E. Cameron, 1995, Nerve function and regeneration in diabetic rats: effects of ZD-7155, an AT_1 receptor antagonist, Am. J. Physiol. 269, E530.

McManis, P.G., P.A. Low and J.K. Yao, 1986, The relationship between nerve blood flow and intercapillary distance in peripheral nerve edema, Am. J. Physiol. 251, E92.

Myers, R.R. and H.C. Powell, 1984, Galactose neuropathy – impact of chronic endoneurial edema on nerve blood flow, Ann. Neurol. 16, 587.

Nagamatsu, M., K.K. Nickander, J.D. Schmelzer, A. Raya, A. Wittrock, H. Tritschler and P.A. Low, 1995, Lipoic acid improves nerve blood flow, reduces oxidative stress and improves distal nerve conduction in experimental diabetic neuropathy, Diabetes Care 18, 1160.

Nukuda, H., 1988, Post-traumatic endoneurial neovascularization and nerve regeneration: a morphometric study, Brain Res. 449, 89.

Nukuda, H., P.J. Dyck and P.A. Low, 1986, Axon caliber and neurofilaments are proportionally decreased in galactose neuropathy, J. Neuropathol. Exp. Neurol. 45, 140.

Powell, H.C., F.M Longo, J. LeBeau and R.R. Myers, 1986, Abnormal nerve regeneration in galactose neuropathy, J. Neuropathol. Exp. Neurol. 45, 151.

Powell, H.C., R.S. Garrett, P.F. Kador and A.P. Mizisin, 1991, Fine structural localization of aldose reductase and ouabain-sensitive K^+-dependent *p*-nitro-phenylphosphatase in rat peripheral nerve, Acta Neuropathol. 81, 529.

Sharma, A.K., S. Bajada and P.K. Thomas, 1981, Influence of streptozotocin-induced diabetes on myelinated nerve fibre maturation and integrity and on body growth in the rat, Acta Neuropathol. 53, 257.

Shupek, M., K.K. Ward, J.D. Schmelzer and P.A. Low, 1989, Comparison of nerve regeneration in vascularized and conventional grafts: nerve electrophysiology, norepinephrine, prostacyclin, malonaldehyde and the blood-nerve barrier, Brain Res. 493, 255.

Tesfaye S., R. Malik and J.D. Ward, 1994, Vascular factors in diabetic neuropathy, Diabetologia 37, 847.

Vinik, A.I., P.G. Newlon, T.J. Lauterio, F.J. Liuzzi, A.S. Depto, G.L. Pittenger and D.W. Richardson, 1995, Nerve survival and regeneration in diabetes, Diabetes Rev. 3, 139.

Williamson, J.R., K. Chang, M. Frangos, K.S. Hasan, Y. Ido, T. Kawamura, J.R. Nyengaard, M. Van Den Enden, C. Kilo and R.G. Tilton, 1993, Hyperglycemic pseudohypoxia and diabetic complications, Diabetes 42, 801.

Yagihashi S., 1995, Pathology and pathogenetic mechanisms of diabetic neuropathy, Diabetes Metab. Rev. 11, 193.