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Nerve function in galactosaemic rats: effects of evening primrose oil and doxazosin

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Abstract

Rats were fed for 6 weeks with a 40% galactose diet to chronically stimulate the polyol pathway. Sciatic motor and saphenous sensory nerve conduction velocity deficits of 22% and 14% respectively developed. Treatment with evening primrose oil or doxazosin from galactosaemia induction partially (approximately 60%) prevented the development of reduced motor and sensory conduction, the former treatment being more successful than the latter. Sciatic nerve resistance to hypoxic conduction failure was 49% increased by galactosaemia. This abnormality was 27% and 43% prevented by doxazosin and evening primrose oil respectively. Galactosaemic sciatic nerves had a 10% increase in water content and endoneurial capillary density was 24% reduced. While neither treatment affected water content, both caused angiogenesis, elevating capillary density by approximately 16%. The data support the hypothesis that, as in experimental diabetes mellitus, the main effect of polyol pathway activation on peripheral nerve function occurs indirectly via a neurovascular action.

Keywords: Polyol pathway; Galactosemia; Nerve conduction; Ischemic resistance; Capillary density; Vasodilator treatment

1. Introduction

Experimental galactosaemia is commonly used to investigate the role of excessive flux through the polyol pathway (Dvornik, 1987). Parallels are drawn with diabetes mellitus where hyperglycaemia 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). Similar dysfunctional states have been found for a variety of tissues in galactosaemic and diabetic models, including peripheral nerve (Calcutt et al., 1994; Cameron and Cotter, 1994a; Cameron et al., 1992; Low and Schmelzer, 1983, Mizisin and Powell, 1993), lens (Dvornik, 1987), retina (Robison et al., 1989), kidney (Dvornik, 1987) and striated muscle (Cotter and Cameron, 1994).

For peripheral nerve, galactosaemia causes reductions in conduction velocity and increased resistance to ischaemic/hypoxic conduction failure that quantitatively closely match findings in experimental diabetes (Cameron et al., 1992; Low and Schmelzer, 1983). There are also distinct differences between the two models in addition to the hypoinsulinaemia which is only present in diabetes. Galactose is metabolized to galactitol by aldose reductase, which parallels the metabolism of glucose to sorbitol in diabetes. However, unlike sorbitol, galactitol is a very poor substrate for sorbitol dehydrogenase (Dvornik, 1987). Thus, the galactosaemic model represents flux through the NADPH-dependent first half of the polyol pathway, whereas diabetes has additional flux through the NAD-dependent second half. Other differences include increased nerve Na^+,K^+-ATPase (ATP phosphohydrolase; EC 3.6.1.3) activity in galactosaemia (Mizisin and Calcutt, 1991) which contrasts with the decrease found in diabetes (Greene et al., 1988), and the presence of nerve oedema in galactosaemia (Cameron et al., 1992; McManis et al., 1986; Mizisin et al., 1986; Myers and Powell, 1984) which does not usually occur in diabetes although it has been occasionally observed (Jakobsen, 1979).

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Recently it has been shown that treatments which do not affect polyol pathway metabolism nevertheless have profound effects on nerve function in diabetes, acting via neurovascular mechanisms. Thus, evening primrose oil provides substrate for eicosanoid synthesis which is depressed in diabetic nerve, and can prevent impaired nerve function, blood flow and endoneurial oxygenation (Cameron and Cotter, 1994a,b; Cameron et al., 1991c; Julu, 1992). Conventional vasodilators, acting on a variety of mechanisms including α_1-adrenoceptors, the renin-angiotensin system, and voltage-gated calcium channels also improve nerve function (reviewed in Cameron and Cotter, 1994a). Given the similarities and differences between experimental diabetes and galactosaemia, the aim was to ascertain whether evening primrose oil or vasodilator treatment could prevent nerve function changes in galactosaemic rats.

2. Materials and methods

All experiments were carried out on mature male Sprague-Dawley rats (Aberdeen University colony), 19 weeks old at the start of the study. One group of non-galactosaemic rats was used as a control. Three other groups were given a 40% galactose diet. This dose was chosen as it causes a level of nerve dysfunction that closely matches that seen in streptozotocin-diabetic rats of this age (Cameron et al., 1991a,1992). One galactosaemic group was untreated for 6 weeks to act as a galactosaemic control. The other galactosaemic groups were treated with either a 10% supplement of evening primrose oil (Scotia Pharmaceuticals, Guildford, Surrey, UK) added to the galactose-enriched chow or with the α_1-adrenoceptor antagonist, doxazosin (Pfizer, Sandwich, Kent, UK), in the drinking water such that the effective dose was approximately 10 mg kg^{-1} day^{-1}. The evening primrose oil dose was chosen as being at the top of the dose-response relationship for correction (< 90%) of nerve conduction velocity deficits in diabetic rats (Dines, K.C., Cameron, N.E. and Cotter, M.A., unpublished observations). For doxazosin, the dose was similar to that of prazosin found effective against reduced nerve conduction velocity and resistance to hypoxic conduction failure in a previous study on diabetic rats (Cameron et al., 1991a).

In final experiments (1–1.5 g kg^{-1} i.p. urethane anaesthesia), nerve conduction velocity was measured in vivo between sciatic notch and knee for motor branches supplying tibialis anterior and gastrocnemius muscles. Sensory conduction velocity was measured in the saphenous nerve between groin and ankle. Methods have been previously described in detail (Cameron et al., 1991c).

Resistance to hypoxic conduction failure was measured in vitro as previously described (Cameron et al., 1991c). Briefly, the sciatic trunk was removed and mounted on bipolar stimulating (proximal) and recording (distal) electrodes. The nerve was equilibrated in Krebs solution at 35°C gassed with 95% O_2/5% CO_2 for 30 min and then transferred to mineral oil pregassed with 100% N_2 for 1 h. Nerves were stimulated with supramaximal pulses (1 Hz, 50 μs width, 10 mA) and myelinated fibre compound action potential amplitude was monitored at 2-min intervals until it was < 10% of the initial value.

Nerve capillarization was estimated from 10 μm frozen sections of sciatic trunk in which the capillary endothelium was stained for alkaline phosphatase, as previously described (Cameron et al., 1991c). Fascicle outlines were traced on a projection microscope and their areas were measured with a digitizing pad linked to a microcomputer to calculate capillary density. For samples from galactosaemic rats, the fascicle was outlined by the area occupied by the nerve fibres rather than that limited by the perineurium. The reason was to avoid biasing the data towards an apparent reduction in capillary density because of subperineurial oedema; the objective being comparison with nerve function, which would primarily be influenced by capillaries in close proximity to nerve fibres within the endoneurium. The correction resulted in an average 9.7% higher capillary density in galactosaemic rats compared to measurements using the perineurium as the outer limit of the nerve fascicle. This figure was not

Table 1
Body weights, sciatic nerve galactitol and myo-inositol concentrations and water content

Group	n	Body weight		Plasma glucose (mmol/l)	Nerve galactitol (ng/g wet weight)	Nerve myo-inositol (ng/g wet weight)	Nerve water content (%)
		Start (g)	End (g)				
Control	10	467 ± 9		8.2 ± 0.4	N.D.	418 ± 24	67.3 ± 0.8
Galactose	12	458 ± 7	451 ± 8	8.9 ± 0.5	2285 ± 185	136 ± 7 [a]	74.1 ± 0.8 [a]
Galactose + doxazosin	11	444 ± 8	420 ± 12	7.6 ± 0.2	2358 ± 63	148 ± 6 [a]	75.4 ± 0.6 [a]
Galactose + EPO	12	439 ± 7	426 ± 6	7.9 ± 0.2	1937 ± 111	212 ± 11 [a,b]	73.4 ± 0.6 [a]

Data are group means ± SEM. EPO: evening primrose oil. N.D. (not detected): below limits of detection in all samples. [a] $P < 0.001$ versus control group, [b] $P < 0.001$ versus galactose-fed group.

altered by treatment. In frozen sections from non-galactosaemic rats, the subperineurial space is comparatively negligible.

Sciatic nerve water content was estimated from samples desiccation-dried to constant weight at 80°C. Nerve galactitol levels were determined by gas chromatography of trimethylsilyl derivatives prepared from aqueous deproteinized extracts (Cameron et al., 1992).

2.1. Statistical analysis

Data are expressed as group means ± S.E.M. One-way analysis of variance was performed, and any significant ($P < 0.05$) differences were assigned to individual between-group comparisons using Student's t-tests, applying the Bonferroni correction for multiple comparisons (Instat, Graphpad, San Diego, CA, USA).

3. Results

Body weights (Table 1) were well maintained in galactosaemic rats and were unaffected by treatment. Plasma glucose levels were also unaltered by galactosaemia. Galactitol accumulated in nerves of galactosaemic rats, but was not detected in the non-galactosaemic group. Galactitol concentrations were unaffected by doxazosin or evening primrose oil treatment. Myo-inositol levels were 67% reduced by galactosaemia and were unaffected by doxazosin treatment. However, evening primrose oil caused an increase in myo-inositol concentration, although this remained 49% reduced compared to the non-galactosaemic group. Nerve water content was increased by approximately 10% with galactosaemia and was unaffected by doxazosin or evening primrose oil treatments.

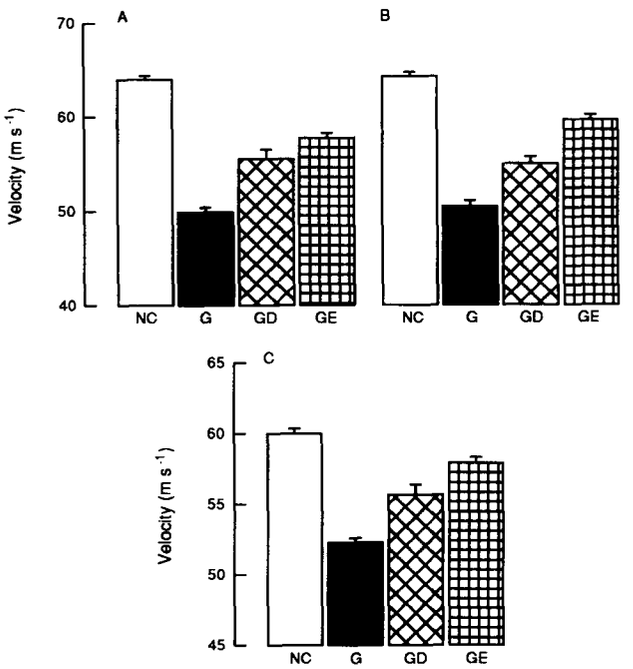

Fig. 1. Conduction velocity values for sciatic branches supplying (A) gastrocnemius and (B) tibialis anterior muscles, and for (C) sensory saphenous nerve. NC, non-galactosaemic control group (open bar), $n = 10$; G, untreated galactosaemic group (filled bar), $n = 12$; GD, 10 mg kg^{-1} day^{-1} doxazosin-treated galactosaemic group (diagonal cross-hatched bar), $n = 11$; GE, evening primrose oil (10% dietary supplement) treated galactosaemic group (cross-hatched bar), $n = 12$. Error bars are S.E.M.

There were 21.5% and 22.2% reductions in sciatic motor nerve conduction velocity to tibialis anterior (Fig. 1A) and gastrocnemius (Fig. 1B) muscles with galactosaemia (both $P < 0.001$). Doxazosin treatment attenuated the development of motor conduction velocity deficits, limiting them to 14.5% and 13.3% ($P < 0.001$ versus non-galactosaemic control and untreated galactosaemic groups) respectively. Evening primrose oil treatment was more effective than doxazosin ($P < 0.01$), with 65.8% and 55.6% prevention of tibialis anterior and gastrocnemius motor conduction velocity deficits respectively ($P < 0.001$ versus non-galactosaemic control and untreated galactosaemic groups). For saphenous sensory conduction (Fig. 1C), the 12.8% galactosaemic deficit was attenuated by doxazosin and evening primrose oil to the extent of 44.0% ($P < 0.001$ versus galactosaemic and non-galactosaemic control groups) and 73.1% ($P < 0.001$ versus galactosaemic group, $P < 0.05$ versus non-galactosaemic control or doxazosin-treated galactosaemic groups) respectively.

In vitro determination of resistance to hypoxic conduction failure (Fig. 2) revealed a prolongation of the decline of compound action potential amplitude with galactosaemia. This was attenuated, but not completely prevented, by doxazosin and evening primrose oil treatments. Thus, the time taken for an 80% reduction in amplitude (T_{80}) was 21.7 ± 0.5 min for non-galactosaemic control nerves. This was increased to 32.4 ± 0.5 min by galactosaemia ($P < 0.001$). With doxazosin treatment, T_{80} was reduced to 29.5 ± 1.0 min ($P < 0.001$ versus non-galactosaemic control, $P < 0.01$ versus galactosaemic groups). Evening primrose oil caused further reduction to 27.9 ± 0.2 min ($P < 0.001$ versus non-galactosaemic control, $P < 0.001$ versus galactosaemic groups). The absolute values for compound action potential amplitude before hypoxia are shown in the inset graph. Compared to non-galactosaemic controls, they were approximately 60% reduced in galactosaemic nerves ($P < 0.001$), regardless of treatment.

Endoneurial capillary density (Fig. 3) was approximately 60 mm^{-2} in non-galactosaemic rats. There was a 24% reduction with galactosaemia ($P < 0.001$) which did not depend on vessel loss as the absolute number of capillaries in the main sciatic fascicle was 47.3 ± 1.1 compared to a control value of 46.4 ± 0.7. Doxazosin

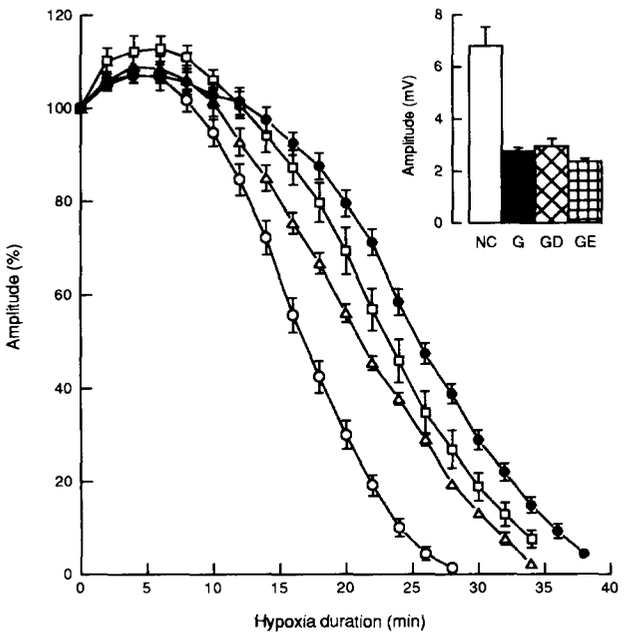

Fig. 2. Sciatic nerve resistance to hypoxia in vitro. Compound action potential amplitude, expressed as a percentage of the initial value plotted against hypoxia duration for control (○), $n = 10$; galactosaemic (●), $n = 12$; 10 mg kg^{-1} day^{-1} doxazosin-treated galactosaemic (□), $n = 11$; and evening primrose oil (10% dietary supplement) treated galactosaemic (△), $n = 12$, groups. Inset graph, initial compound action potential amplitudes, NC, non-galactosaemic control; G, untreated galactosaemic; GD, doxazosin-treated galactosaemic; GE, evening primrose oil-treated galactosaemic group. Error bars are S.E.M.

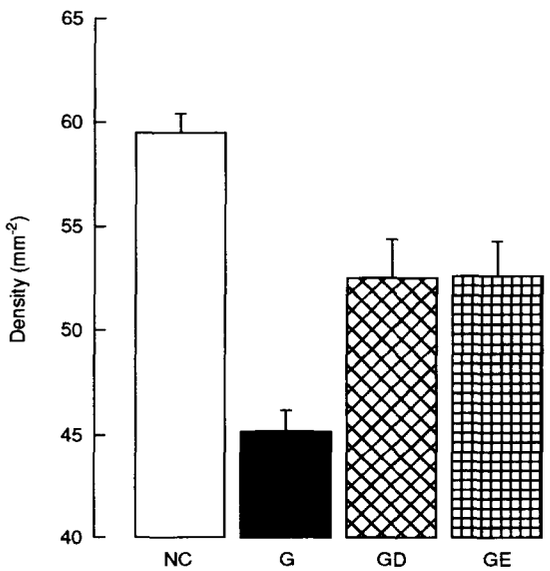

Fig. 3. Sciatic endoneurial capillary density, measured in frozen sections stained for alkaline phosphatase. NC, non-galactosaemic control group (open bar), $n = 10$; G, untreated galactosaemic group (filled bar), $n = 12$; GD, 10 mg kg^{-1} day^{-1} doxazosin-treated galactosaemic group (diagonal cross-hatched bar), $n = 11$; GE, evening primrose oil (10% dietary supplement) treated galactosaemic group (cross-hatched bar), $n = 12$. Error bars are S.E.M.

treatment resulted in an increase of 16.2% compared to untreated galactosaemia ($P < 0.01$), which was not sufficient to normalize capillary density ($P < 0.01$). Treatment with evening primrose oil caused a similar 16.4% increase ($P < 0.01$) which remained reduced compared to non-galactosaemic rats ($P < 0.01$). In treated and untreated galactosaemia there was a positive correlation between capillary density and motor conduction velocity ($r = 0.424$, $P = 0.012$), and a negative correlation with T_{80} ($r = -0.516$, $P = 0.0025$).

4. Discussion

The data show that galactosaemia causes conduction velocity deficits and resistance to hypoxic conduction failure, in agreement with the literature (Cameron et al., 1992; Low and Schmelzer, 1983). This is the first demonstration that two treatments which are effective in experimental diabetes, evening primrose oil (Cameron et al., 1991c; Dines et al., 1993; Julu, 1992) and α_1-adrenoceptor inhibition (Cameron et al., 1991a), reduce galactosaemic nerve dysfunction without altering polyol pathway activity, assessed by galactitol levels. In contrast, evening primrose oil and α_1-adrenoceptor antagonists do not affect nerve function in normal rats (Cameron et al., 1991a; Dines et al., 1993).

The causes of nerve dysfunction in galactosaemia and diabetes are the subject of considerable discussion. Both neurochemical and vascular explanations have been proposed. Thus, the reduction in myo-inositol consequent on polyol pathway activity, noted in this and other studies (Dvornik, 1987), may be responsible for decreased phosphoinositide turnover, reduced stimulation of protein kinase C and hence diminished neuronal Na^+,K^+-ATPase activity. The latter has been suggested to cause reduced conduction velocity, increased resistance to hypoxic conduction failure and a series of changes resulting in axonal degeneration in diabetes (Greene et al., 1988). Recent investigations have, however, cast doubt on this hypothesis for diabetes and galactosaemia. Thus, in diabetes, nerve function may be corrected by vasodilator treatment which does not affect the diminished myo-inositol concentration or Na^+,K^+-ATPase activity (Cameron et al., 1991a). The link between correction of Na^+,K^+-ATPase deficits and polyol pathway inhibition has been questioned (Sredy et al., 1991) and in rats fed a 40% galactose diet Na^+,K^+-ATPase activity is doubled (Mizisin and Calcutt, 1991) rather than reduced, despite myo-inositol lowering. The myo-inositol effect, therefore, appears to be an epi-phenomenon as far as nerve conduction is concerned. It probably reflects a role as a Schwann cell osmolyte, being reduced as galactitol or sorbitol concentration increases (Hohman and Carper, 1990). The partial correction of conduc-

tion velocity by doxazosin without any effect on myo-inositol in galactosaemia also argues against the relevance of this mechanism. There was, however, a small increase in nerve myo-inositol with evening primrose oil treatment. This would not be expected to affect conduction velocity as the deficit still remained greater than in diabetic rats (Dvornik, 1987), and dietary myo-inositol supplementation sufficient to normalize nerve levels does not alter the diabetic conduction deficit (Cameron et al., 1994b). The effect of evening primrose oil on nerve myo-inositol perhaps relates to altered neuronal phosphoinositide turnover (Julu, 1992).

An alternative neurovascular hypothesis is more likely to account for nerve deficits in galactosaemia and diabetes. Thus, in diabetes nutritive blood flow is reduced and this results in endoneurial hypoxia (Tuck et al., 1984). Prevention of the nutritive perfusion deficit using vasodilators, evening primrose oil, or by metabolic intervention using aldose reductase inhibition, anti-oxidants, or aminoguanidine which blocks the formation of advanced glycation products, also prevents nerve dysfunction in experimental diabetes (Cameron and Cotter, 1994a,b; Cameron et al., 1991b, 1994a,b; Kihara et al., 1991). In galactosaemia, endoneurial oxygenation is also impaired. This appears to have two causes: nerve blood flow may be diminished (Myers and Powell, 1984) and the situation is exacerbated by oedema. The latter is particularly seen in the subperineurial space and around capillaries. It depends on both galactitol accumulation and increased endoneurial Na^+ concentration (Mizisin et al., 1986), the latter perhaps resulting from Na^+-galactose co-transport across the blood-nerve barrier. The resultant elevation of intercapillary spacing increases oxygen diffusion distances, hence reducing oxygen supply to neurones and Schwann cells (McManis et al., 1986). The 22.6% decrease in apparent capillary density without a change in absolute vessel number of the main sciatic fascicle in galactosaemia concurs with this suggestion and approximates the 20.8% decrease in the proportion of nerve that is not water.

Treatment with doxazosin and evening primrose oil elevated capillary density to a similar extent. However, the effects of evening primrose oil on conduction velocity were greater than those of doxazosin, suggesting that another mechanism besides partial correction of an oedema-mediated increase in intercapillary distance was also in operation. This may relate to an accompanying blood flow deficit (Myers and Powell, 1984) caused by changes in resistance vessels. The involvement of polyol pathway activity in diminished endothelium-dependent nitric oxide (NO)-mediated relaxation of large vessels has been documented for both diabetes and galactosaemia (Cameron and Cotter, 1992, 1993; Cohen, 1993). In addition, polyol pathway flux appears to inhibit vessel prostacyclin production, which is restored by aldose reductase inhibition in aortas from diabetic rats (Wakasugi et al., 1991). Aldose reductase is localised in small arteries and arterioles of peripheral nerve (Powell et al., 1991), therefore, these mechanism probably contribute to reduced nerve blood flow in diabetes (Cameron and Cotter, 1994b; Cameron et al., 1994b) and galactosaemia. Endothelial dysfunction is linked to increased oxygen free radical activity (Cameron et al., 1994a; Cohen, 1993), which partly result from auto-oxidation reactions and the formation of advanced glycation end products that are accelerated by elevated sugar levels in diabetes and galactosaemia (Baynes, 1991). Hyperglycaemia may also impair endogenous free radical scavenging mechanisms, causing a reduction in endothelial cell and sciatic nerve glutathione levels (Tagami et al., 1992; Nickander et al., 1994) which may be due to enhanced polyol pathway flux and competition between aldose reductase and glutathione reductase for NADPH (Cameron and Cotter, 1994a; Cohen, 1993). Abnormalities of endothelium function increase vasa nervorum reactivity to vasoconstrictors (Maxfield et al., 1994), and elevate the synthesis/release of angiotensin II and endothelin-1 (Cameron and Cotter, 1994a). It is plausible that doxazosin had a lesser effect than evening primrose oil because it blocked only one process, α_1-adrenoceptor-mediated vasoconstriction. Evening primrose oil would be expected to have a multiple action as a substrate for local prostacyclin production, which would both cause vasodilation and, via receptor-mediated mechanisms, offset the effects of more than one class of endogenous vasoconstrictor. Doxazosin lowers total plasma cholesterol in patients (Pool, 1991). The blood cholesterol status is not known in galactosaemic rats but such an effect is unlikely to have influenced nerve function in this investigation because of the relatively short time course of the experiments. In the long term, a cholesterol-lowering action could perhaps contribute to improved vascular performance because of reduced atherogenesis. Prevention of conduction velocity deficits and increased resistance to hypoxic conduction failure in diabetic rats by similar doses of evening primrose oil and an α_1-adrenoceptor antagonist were somewhat greater than in galactosaemia (Cameron et al., 1991a,c). The difference may perhaps be attributed to the incomplete restoration of intercapillary distance by treatment in galactosaemia because of the oedema which is not a prominent feature in diabetes. This would limit oxygen supply to axons and Schwann cells despite elevated blood flow.

The finding of increased vessel density in treated galactosaemic rats, albeit against a background of oedema, parallels changes in diabetes for both evening primrose oil and α_1-adrenoceptor blockade (Cameron et al., 1991a,c). The main stimulus for angiogenesis is the mechanical effect of a chronic blood flow elevation

on vascular endothelium (Hudlická and Tyler, 1986). The fact that the magnitudes of capillary increases were similar for the two treatments suggests that any drug-induced cardiovascular changes that could potentially affect nerve blood flow were also similar. However, chronic flow elevation is not the only important factor for angiogenesis. Although noradrenergic inhibition increased blood flow in normal rats, vasa nervorum capillary growth was not seen (Cameron et al., 1991a,b). This suggest that galactosaemia and diabetes are pro-angiogenic. In both experimental models there is a deficit in NO, which is an anti-angiogenic agent (Pipili-Synetos et al., 1994). Treatments that elevate blood flow in diabetes by an action to improve the NO system, such as aldose reductase inhibitors, anti-oxidants, aminoguanidine, or nitrovasodilators do not increase endoneurial capillarization (Cameron and Cotter, 1994a, 1995; Kihara et al., 1991). In contrast, treatments that produce similar blood flow elevations in diabetes but are unlikely to correct the NO deficit, such as evening primrose oil, prostacyclin analogues, α_1-adrenoceptor inhibitors, and angiotensin II antagonists, cause angiogenesis (Cameron and Cotter, 1994a; Cameron et al., 1991a,c; Cotter et al., 1993). Thus, the lack of anti-angiogenic action by NO could be partly responsible for the elevated angiogenic potential in diabetes and galactosaemia. It is not known whether this mechanism contributes to other vascular complications, for example vessel proliferation in retinopathy.

Mechanisms underlying elevated hypoxic resistance are not known in detail. For diabetes, hypoxic resistance is caused by increased reliance on anaerobic metabolism, as a chronic adaptation to endoneurial hypoxia (Low et al., 1986). Grafe and co-workers, however, showed that several hours exposure of nerves to hyperglycaemia in vitro increases anaerobic metabolism, and suggested hyperglycaemia as a direct stimulus for the development of resistance to hypoxic conduction failure in vivo (Schneider et al., 1993). In their experiments the effect was glucose-specific, other sugars like galactose being ineffective. The hypoxic resistance with chronic galactosaemia was profound, and very similar in magnitude to that seen after a comparable period of diabetes (Cameron et al., 1992). It is likely that reduced perfusion, coupled with increased intercapillary distance are the main causes of the development of resistance to hypoxic conduction failure in galactosaemia, partial correction by evening primrose oil and doxazosin paralleling findings in diabetic rats (Cameron et al., 1991a,c; Cameron and Cotter, 1993; Dines et al., 1993). Furthermore, increased capacity for anaerobic energy metabolism would be necessary to combat the greater energy demand of Na^+,K^+-ATPase activity associated with altered endoneurial electrolytes in galactosaemia (Mizisin et al., 1986). This contrasts with the lowering of Na^+,K^+-ATPase in diabetes, which may represent a mechanism for energy saving in the face of reduced oxygen supply (Cameron and Cotter, 1994a) that is not possible in galactosaemia. The reduction of initial compound action potential amplitude in galactosaemic rats probably results from subperineurial oedema rendering the impulse generation site distant from the recording electrodes.

In conclusion, the diabetes-like changes in nerve function in galactosaemic rats are partially prevented by evening primrose oil and vasodilator treatments. The data support a neurovascular hypothesis of the aetiology of galactosaemic complications, and the role of polyol pathway hyperactivity as a potential cause of vascular disease. They suggest that galactosaemia is a good model for aspects of diabetic neuropathy and microangiopathy, with the potential simplification of normal insulin levels and lack of severe body wasting compared to experimental diabetes (Dvornik, 1987). It remains to be seen whether the treatments used in this investigation, which do not alter polyol pathway flux, could nevertheless protect galactosaemic Schwann cells and axons from long-term morphological damage (Mizisin and Powell, 1993) or whether, unlike the nerve function changes, these depend on osmotic imbalances caused by polyol accumulation.

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