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Dissociation between biochemical and functional effects of the aldose reductase inhibitor, ponalrestat, on peripheral nerve in diabetic rats

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1 The aim of the study was to examine the effects in rats of two different doses of the aldose reductase inhibitor, ponalrestat, on functional measures of nerve conduction and sciatic nerve biochemistry.

2 After 1 month, streptozotocin-induced diabetes produced 22%, 23% and 15% deficits in conduction velocity of sciatic nerves supplying gastrocnemius and tibialis anterior muscles and saphenous sensory nerve respectively compared to controls. These deficits were maintained over 2 months diabetes.

3 Slower-conducting motor fibres supplying the interosseus muscles of the foot did not show a diabetic deficit compared to onset controls, however, there was a 13% reduction in conduction velocity after 2 months diabetes relative to age-matched controls, indicating a maturation deficit.

4 Resistance to hypoxic conduction failure was investigated for sciatic nerve trunks *in vitro*. There was an increase in the duration of hypoxia necessary for an 80% reduction in compound action potential amplitude with diabetes. This was progressive; after 1 month, hypoxia time was increased by 22% and after 2 months by 57%.

5 The effect of 1-month treatment with the aldose reductase inhibitor, ponalrestat, on the abnormalities caused by an initial month of untreated diabetes was examined. Two doses of ponalrestat were employed, 8 mg kg^{-1} day^{-1} (which is equivalent to, or greater than, the blockade employed in clinical trials), and 100 mg kg^{-1} day^{-1}.

6 Sciatic nerve sorbitol content was increased 7 fold by diabetes. Both doses were effective in reducing this; 70% for 8 mg kg^{-1} day^{-1}, and to within the control range for 100 mg kg^{-1} day^{-1}. However, 8 mg kg^{-1} day^{-1} produced only a modest lowering (44%) of the 8 fold increase in fructose content, indicating that flux through the polyol pathway remained substantially elevated. For 100 mg kg^{-1} day^{-1} ponalrestat, fructose content was within the normal range, indicating a profound inhibition of flux through the pathway.

7 Conduction velocity abnormalities in sciatic motor branches supplying gastrocnemius and tibialis anterior muscles, and sensory saphenous nerve were completely restored by treatment with ponalrestat at 100 mg kg^{-1} day^{-1}, whereas 8 mg kg^{-1} day^{-1} was completely ineffective. The maturation deficit for interosseus motor nerve was unaffected by treatment.

8 Neither 8 or 100 mg kg^{-1} day^{-1} ponalrestat reversed the increased resistance to hypoxic conduction failure resulting from the initial month of untreated diabetes. However, both doses prevented further increases in hypoxic resistance over the treatment period.

9 Three main conclusions were reached. First, substantial blockade of polyol pathway flux is necessary to reverse conduction velocity deficits and this degree of aldose reductase inhibition has not been achieved in clinical trials. Second, nerve content of fructose is a better biochemical indicator of likely functional benefit than that of sorbitol. Third, conduction velocity and hypoxic resistance were differentially affected by the two doses of ponalrestat, a finding that suggests differences in their aetiology.

Keywords: Neuropathy; nerve conduction; ischaemia; aldose reductase; polyol pathway; sorbitol; streptozotocin-induced diabetes; ponalrestat

Introduction

Defects in nerve function in diabetes have been linked to a hyperglycaemia-related increase in polyol pathway activity. Glucose is converted to the sugar alcohol, sorbitol, by the first pathway enzyme, aldose reductase, and sorbitol is subsequently metabolized to fructose by sorbitol dehydrogenase (Dvornik, 1987). It has been suggested that polyol pathway activation is responsible for a decrease in nerve *myo*-inositol concentration, leading to reduced Na-K ATPase pump activity (Green *et al.*, 1985) but substantial doubt has been cast over the obligatory involvement of a Na-K ATPase deficit in diabetic neuropathy (Bianchi *et al.*, 1987). Although several studies in animal models have demonstrated that aldose reductase inhibitors (ARIs) can prevent the slowing of nerve conduction characteristic of early diabetes (Mayer & Tomlinson, 1983; Cameron *et al.*, 1986a,b), their efficacy in either preventing deficits in the longer term (Willars *et al.*, 1988) or correcting established abnormalities has been questioned (Cameron *et al.*, 1989). Furthermore, clinical trials of ARIs have demonstrated only very modest benefits with respect to objective measures such as nerve conduction velocity (NCV), although the regeneration of damaged nerve fibres may be improved (Sima *et al.*, 1988). There are a number of potential explanations for the discrepancy between animal studies and clinical trials. Animal models may be unsuitable; the duration of diabetes is generally much shorter than in patients, there is less overt fibre damage in rat nerves than in biopsy samples from neuropathic patients, and, since they are much longer than those in rats, human nerves may, therefore, be more vulnerable to peripheral vascular disease. However, one factor that clearly differs between rat and human studies is the dose of ARIs employed, generally an order of magnitude

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greater in the former. Thus, the main aims of this investigation were to ascertain whether a dose of an ARI similar to the upper limit used in the clinical trials was effective in diabetic rats, and to gauge what level of polyol pathway blockade might be needed to correct established conduction deficits.

In addition to reduced NCV, nerves in diabetic patients and animal models show an increased resistance to ischaemic conduction failure (RICF). This may be partially prevented by ARI treatment (Price *et al.*, 1988), although involvement of the polyol pathway in the aetiology of this abnormality has been disputed (Jaramillo *et al.*, 1984; Carrington *et al.*, 1991). An additional aim was to investigate this phenomenon.

Methods

Male Sprague-Dawley rats (Aberdeen University breeding colony), 19 weeks old at the start of the study were used. One group of non-diabetic animals acted as onset controls. Another was studied 2 months later, acting as age-matched controls. Others were given streptozotocin (45 mg kg^{-1} in 20 mmol l^{-1} sodium citrate buffer, pH 4.5, i.p.). Diabetes was verified 24 h later by estimating hyperglycaemia and glycosuria (Visidex II and Diastix; Ames, Slough). Animals were tested weekly, and weighed daily. They were rejected if blood glucose concentration was <20 mmol l^{-1} or if they showed a consistent increase in body weight over 3 days. Samples for plasma glucose measurement were also taken the day of final experiments.

Diabetic animals were divided into 4 groups. Two were untreated, acting as diabetic controls, and were studied after 1 or 2 months. Two further groups were left untreated for 1 month and were then given ponalrestat (Stribling *et al.*, 1985) either 8 or 100 mg kg^{-1} day^{-1} orally for a further month. The lower dose was chosen to be similar to the highest dose of ponalrestat (600 mg day^{-1}) given to patients in clinical trials (Florkowski *et al.*, 1991).

In final experiments (1–1.5 g kg^{-1} urethane anaesthesia i.p.), NCV was measured *in vivo* between the sciatic notch and knee for motor branches supplying tibialis anterior (peroneal division) and gastrocnemius (proximal tibial division) muscles and the interosseous muscle of the foot (distal tibial division). NCV in sensory nerves was measured in the saphenous nerve between groin and ankle. Methods have previously been described in detail (Cameron *et al.*, 1989).

RICF was measured *in vitro* (Cameron *et al.*, 1991b) after the NCV measurements. The contralateral sciatic trunk was removed and mounted on bipolar stimulating (proximal end) and recording (distal end) electrodes in a chamber filled with Krebs solution (composition, mM: Na^+ 144.0, K^+ 5.0, Ca^{2+} 2.5, Mg^{2+} 1.1, HCO_3^- 25.0, PO_4^{2-} 1.1, SO_4^{2-} 1.1) at 35°C containing 5.5 mmol l^{-1} glucose for nerves from non-diabetic rats and 40 mmol l^{-1} glucose for the diabetic groups. Previous experiments (Cameron, Cotter & D. Cox, unpublished observations) have shown that varying glucose concentration between 5.5 and 40 mmol l^{-1} does not have a significant effect on nerve hypoxic resistance under these conditions. Bathing fluid was gassed with 95% O_2:5% CO_2 (pH 7.35). Nerves were equilibrated for 30 min, then the chamber was refilled with mineral oil pregassed with 100% N_2 for 1 h, and N_2 gassing continued. Nerves were stimulated with just supramaximal pulses (1 Hz, 0.05 ms width, 10 mA) and compound action potential amplitude was monitored at 2 min intervals until it fell below 10% of its initial value. Sciatic nerves used for NCV measurements were rapidly dissected out before rats were killed. They were frozen in liquid nitrogen and then stored at −80°C. Nerve sugars and polyol concentrations were subsequently determined by gas chromatography of trimethyl-silyl derivatives prepared from aqueous deproteinized extracts (Stribling *et al.*, 1985).

Data are expressed as means ± s.e.mean. One-way analysis of variance was performed, followed by the Bonferroni corrected t test to assign differences to individual between-group comparisons when overall significance ($P<0.05$) was attained, using commercial software (Instat, Graphpad, San Diego, CA, U.S.A.).

Drugs

Streptozotocin and urethane were obtained from Sigma and ponalrestat was a gift from I.C.I. Pharmaceuticals.

Results

Body weights and final plasma glucose levels for control and diabetic rats are given in Table 1. Diabetes resulted in progressive weight loss, which after 2 months, was about 25%: controls showed a 16% weight gain over this period. Plasma glucose concentration was elevated approximately 5 fold by diabetes. Ponalrestat treatment at 8 and 100 mg kg^{-1} day^{-1} had no significant effect on these parameters.

Motor NCV results for tibialis anterior and gastrocnemius muscles are shown in Figure 1a and b respectively. There were no significant differences between onset and age-matched non-diabetic control groups. There was a decline in NCV for both nerve branches of around 22% over the first month of untreated diabetes, reaching 25% at 2 months ($P<0.001$ at both time points, for both nerves). When ponalrestat treatment was given to reverse the initial deficit, there was no significant effect with a dose of 8 mg kg^{-1} day^{-1}, but reversal was complete with 100 mg kg^{-1} day^{-1} ($P<0.001$ for both nerves).

Unlike the more proximal motor branches, the interosseous nerve was slower conducting, but increased during the experimental period such that age-matched NCV was greater by 13% than onset controls ($P<0.05$) (Figure 1c). This increase was halted by 2 months' diabetes ($P<0.01$) but there was no significant deficit compared to the onset control level. NCV was not restored to age-matched control values by ponalrestat at either dose level ($P<0.01$) and remained not significantly different from the onset control value. This contrasts with NCV changes in sensory saphenous nerves (Figure 1d) which showed a pattern similar to fast conducting motor nerves; with no significant difference between control groups, a 12% deficit with two months diabetes ($P<0.001$ compared to onset controls) and complete restoration by 100 ($P<0.001$) but not 8 mg kg^{-1} day^{-1} ponalrestat.

Figure 2 illustrates the data from RICF measurements. Initial compound action potential amplitudes are shown in the inset graph and did not differ significantly between groups. When hypoxic, the nerves of onset and age-matched control groups showed a rapid depression of compound action potential amplitude, after a short period of hyperexcitability (Seneviratne & Peiris, 1969). This decline was relatively prolonged in preparations from 1-month diabetic controls and more so after 2 months. Curves for the ponalre-

Table 1 Body weights and plasma glucose concentrations in control and diabetic rats

Group	n	*Weight* (g) *Start*	*Finish*	*Glucose* (mmol l^{-1})
Controls				
Onset	20	484 ± 12	–	6.4 ± 0.3
Age-matched	12	507 ± 19	589 ± 20	8.0 ± 0.5
Diabetic				
1 month	10	461 ± 6	413 ± 12	33.0 ± 3.1
2 month	20	514 ± 11	377 ± 11	40.6 ± 2.4
Ponalrestat-treated				
8 mg kg^{-1} day^{-1}	11	473 ± 9	357 ± 8	35.9 ± 2.2
100 mg kg^{-1} day^{-1}	11	481 ± 10	346 ± 14	38.3 ± 2.7

Data are means ± s.e.mean.

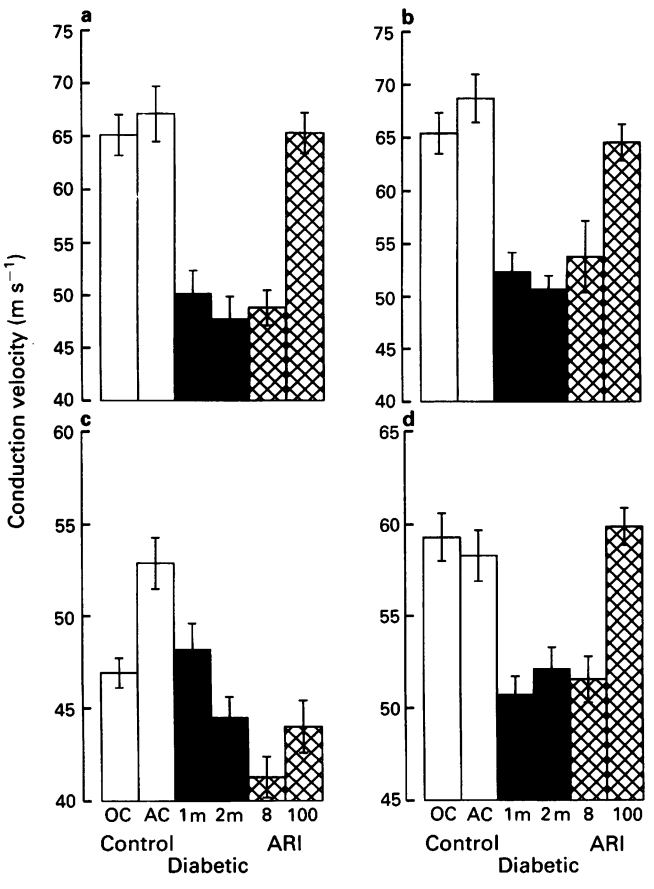

Figure 1 Conduction velocity in motor and sensory nerves for control diabetic and ponalrestat-treated diabetic rats: (a) tibialis anterior; (b) gastrocnemius; (c) interosseus muscles; (d) sensory saphenous nerve. Columns show means (± s.e.means, vertical bars). Controls (open columns): OC, onset controls ($n = 20$); AC, age-matched controls ($n = 12$); diabetic (closed columns), 1 month ($n = 10$), 2 month ($n = 20$); ARI, ponalrestat-treated diabetic (cross-hatched columns), 8 mg kg^{-1} day^{-1} ($n = 11$), 100 mg kg^{-1} day^{-1} ($n = 11$). For tibialis anterior, gastrocnemius and saphenous nerves, values were significantly reduced by 1 and 2 months' untreated diabetes compared to onset or age-matched controls ($P < 0.001$, all comparisons). In ponalrestat-treated diabetic groups, 8 mg kg^{-1} day^{-1} had no significant effect on conduction velocity whereas 100 mg kg^{-1} day^{-1} completely restored conduction ($P < 0.001$, compared to 2-month diabetic controls). For interosseous nerve, conduction velocity in 2 month diabetic controls was reduced compared to age-matched ($P < 0.01$) but not to onset controls. Ponalrestat treatment did not have a significant effect.

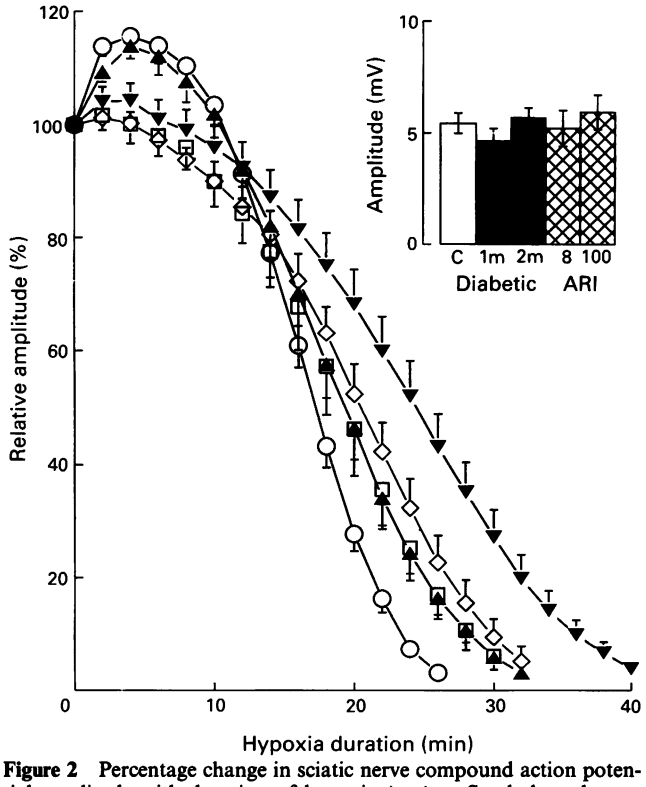

Figure 2 Percentage change in sciatic nerve compound action potential amplitude with duration of hypoxia *in vitro*. Symbols and error bars show group means ± s.e.means. Non-diabetic control, onset and age-matched controls pooled for clarity (○); diabetic control 1-month (▲), 2-month (▼); ponalrestat-treated diabetic groups, 8 mg kg^{-1} day^{-1} (◇), 100 mg kg^{-1} day^{-1} (□). The inset histogram shows initial sciatic nerve compound action potential amplitudes before the period of hypoxia for non-diabetic controls (C, open column bar), 1-month and 2-month diabetic controls (solid columns); and 8 mg kg^{-1} day^{-1} and 100 mg kg^{-1} day^{-1} ponalrestat-treated diabetic (cross-hatched columns) groups. There were no significant between-group differences in initial amplitude.

stat-treated rats lay close to that for the 1 month diabetic controls. This is reflected by the times taken for an 80% reduction in compound action potential amplitude, plotted as a histogram in Figure 3. It shows the progressive nature of the phenomenon with diabetes duration ($P < 0.01$ comparing 1 month diabetes with onset controls, $P < 0.001$ comparing 1- and 2-month diabetic groups). There was a good agreement between the 1-month diabetic controls and the treated groups, both of which were significantly different from the 2 months group ($P < 0.01$ for 8 mg kg^{-1} day^{-1}, $P < 0.001$ for 100 mg kg^{-1} day^{-1}). Thus, both levels of treatment prevented a further increase in hypoxic resistance, but neither reversed the initial deficit ($P < 0.001$ and $P < 0.01$ compared to onset controls for 8 mg kg^{-1} day^{-1} and 100 mg kg^{-1} day^{-1} respectively).

Sciatic nerve polyol concentrations are shown in Table 2. There were no significant differences between control groups so they have been pooled. Similarly, there were no differences between diabetes of 1 or 2 months' duration. With diabetes,

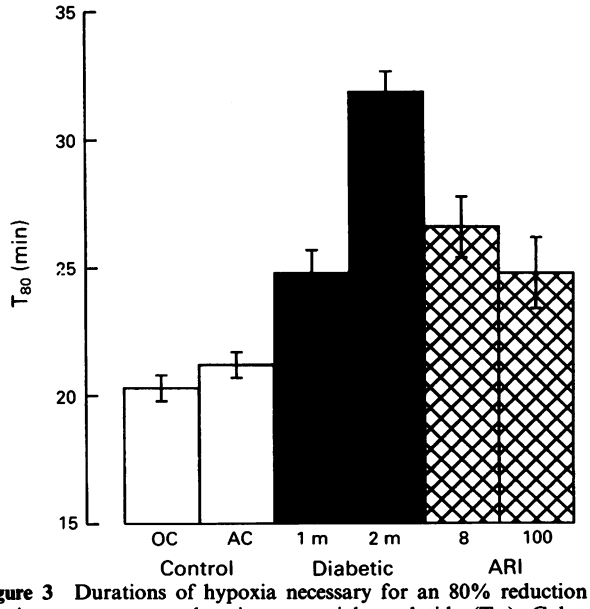

Figure 3 Durations of hypoxia necessary for an 80% reduction in sciatic nerve compound action potential amplutide (T_{80}). Columns show means (± s.e.means, vertical bars). Controls (open columns); OC, onset controls ($n = 20$); AC, age-matched controls ($n = 12$); diabetic (solid columns), 1 m, 1-month ($n = 10$); 2 m, 2-month ($n = 20$); ARI, ponalrestat-treated diabetic (cross-hatched columns), 8 mg kg^{-1} day^{-1} ($n = 11$), 100 mg kg^{-1} day^{-1} ($n = 11$). Untreated diabetes caused a progressive increase in T_{80} ($P < 0.01$ for 1-month group; $P < 0.001$ for 2-month group compared to onset controls). Treatment with 8 and 100 mg kg^{-1} day^{-1} ponalrestat prevented further increases in T_{80} between 1 and 2 months ($P < 0.001$ and $P < 0.001$ respectively compared to the 2-month diabetic group).

Table 2 Sciatic nerve polyol pathway metabolite and *myo*-inositol concentrations in control and diabetic rats

Group	n	*Sorbitol*	*Fructose*	myo-*Inositol*
Control	29	0.282 ± 0.022	0.898 ± 0.069	3.874 ± 0.342
Diabetic	27	1.979 ± 0.129[b]	7.595 ± 0.509[b]	2.067 ± 0.086[b]
Ponalrestat-treated				
8 mg kg^{-1} day^{-1}	11	0.725 ± 0.101[d]	4.634 ± 0.637[b,d]	2.710 ± 0.210[a]
100 mg kg^{-1} day^{-1}	11	0.104 ± 0.008[d,e]	0.428 ± 0.037[d,f]	3.160 ± 0.230[c]

Data are means ± s.e.means, expressed as nmol mg^{-1} nerve wet weight.
[a]$P<0.05$; [b]$P<0.001$: versus control group.
[c]$P<0.05$; [d]$P<0.001$: effect of ponalrestat treatment versus diabetic group.
[e]$P<0.05$; [f]$P<0.001$: effect of level of ponalrestat treatment, 100 mg kg^{-1} day^{-1} versus 8 mg kg^{-1} day^{-1}.

sorbitol concentration was increased 7 fold, and fructose showed a corresponding 8 fold elevation; 8 mg kg^{-1} day^{-1} ponalrestat reduced the excess sorbitol levels by 70%, but had a lesser effect (44%) on fructose concentration. By contrast, the 100 mg kg^{-1} day^{-1} dose reduced both sorbitol and fructose to within or below the control concentration range. Nerve *myo*-inositol concentration was decreased by 47% in diabetic animals; although the deficit was ameliorated to the extent of 60% with 100 mg kg^{-1} day^{-1} it was not significantly affected by treatment at 8 mg kg^{-1} day^{-1}.

Discussion

The data demonstrate two main points. First, a minor degree of polyol pathway inhibition (8 mg kg^{-1} day^{-1} ponalrestat), which largely blocked sorbitol accumulation but did not substantially reduce fructose concentration, also did not restore changes in motor or sensory NCV in diabetic rats. When the nerve fructose concentration was normalized by treatment with the high dose (100 mg kg^{-1} day^{-1}) of ponalrestat, indicating a substantial inhibition of polyol pathway flux, NCV was restored. In previous studies, we used an intermediate dose of ponalrestat (25 mg kg^{-1} day^{-1}) and found sorbitol concentrations were normal, fructose was somewhat elevated, and NCV changes only partially reversed (Cameron *et al.*, 1989). Taken together, this suggests that a high degree of pathway blockade is necessary for optimal effects on NCV, and that fructose concentration provides a better biochemical indicator than sorbitol concentration for potential functional improvements. Second, different measures of nerve function show differential sensitivity to polyol pathway inhibition since the low dose ARI had no effect on NCV whilst largely preventing a further increase in RICF.

In clinical trials, regardless of the ARI employed, polyol pathway inhibition was no better than found in rats with 8 mg kg^{-1} day^{-1} ponalrestat. For example, reductions of erythrocyte or nerve biopsy sorbitol concentration of about 50% have been reported (reviewed in Dvornik, 1987). This suggests a fairly low degree of blockade of the pathway, probably insufficient to test adequately the hypothesis that enhanced polyol pathway flux makes a major contribution to the aetiology of diabetic neuropathy. Thus, it is likely that the failure to find significant improvements in clinical trials of ponalrestat (Florkowski *et al.*, 1991) reflects the use of a drug dose that did not produce adequate inhibition of aldose reductase.

The lack of effect of ponalrestat (8 or 100 mg kg^{-1} day^{-1}) on interosseous NCV agrees with a previous finding with 25 mg kg^{-1} day^{-1} (Cameron *et al.*, 1989). The NCV deficit arises from comparison with age-matched rather than onset controls and can largely be explained by lack of nerve growth resulting in small diameter fibres that have a normal NCV for their size (Cameron *et al.*, 1986b). Thus, ponalrestat treatment cannot restore normal nerve growth in our experimental model. Other workers have found increases in interosseous NCV with ARI treatment, but usually in younger rats in a more rapid growth phase (Gillon *et al.*, 1983; Mayer & Tomlinson, 1983). In addition, methodological considerations may explain these conflicting results. Concentric bipolar recording electrodes were used in this investigation to ensure focal recording. In the other studies unipolar needle electrodes were used, thus, contamination by potentials from nearby muscles whose nerves respond more like gastrocnemius or tibialis anterior to treatment cannot be excluded.

The cause of increased RICF in diabetic nerves is disputed, there being two main schools of thought. According to the metabolic hypothesis, nerves are more resistant to ischaemia because a major requirement for ATP is to supply the Na-K ATPase pump. Na-K ATPase activity is reduced by around 50% in homogenates from diabetic nerves compared to controls (Das *et al.*, 1976; Lambourne *et al.*, 1988; Cameron *et al.*, 1991c). Diabetic nerves could, therefore, utilize energy stores more slowly and maintain function longer when oxidative metabolism is prevented. ARIs would be expected to prevent increased RICF as treatment improves Na-K ATPase activity (Greene *et al.*, 1985), although this has been disputed (Lambourne *et al.*, 1988), the contradictory results being explained by differences in dietary composition and measurement procedure (Sredy *et al.*, 1991). A suggested mechanism is that a diabetic deficit in nerve *myo*-inositol, as noted in this study, leads to reduced membrane phosphoinositide turnover, and less diacylglycerol-mediated activation of protein kinase C, which in turn increases Na-K ATPase activity (Greene *et al.*, 1985). ARI-treatment tends to restore *myo*-inositol levels, as noted for the 100 mg kg^{-1} day^{-1} dose in this study; therefore, it may be expected to restore Na-K ATPase activity.

The same polyol-pathway-dependent Na-K ATPase deficit has also been suggested to explain reduced NCV (Greene *et al.*, 1985). Thus, NCV and RICF changes should occur in parallel, whereas they were dissociated between the two ARI doses in this study. The time-course for NCV changes and RICF development also differ. NCV deficits develop over the first 2 weeks of diabetes, and have virtually reached asymptote by 1 month, with little further change to 4 months (Cameron *et al.*, 1989). In contrast, RICF shows a fairly linear increase with time, as noted in the present study for 1 and 2 months. In addition, other findings are at variance with this metabolic hypothesis. RICF was improved by 8 mg kg^{-1} day^{-1} ponalrestat, whereas *myo*-inositol levels were not significantly affected. In normal rats fed a galactose-enriched diet, the polyol pathway is stimulated (Dvornik, 1987); however, rather than reducing sciatic nerve Na-K ATPase activity, it is doubled (Lambourne *et al.*, 1988). This is accompanied by NCV and RICF abnormalities very similar to those noted for experimental diabetes (Low & Schmelzer, 1983; Cameron *et al.*, 1992), whereas the prediction is that these parameters would be normal or even supranormal.

An alternative vascular hypothesis better explains the data. Sciatic blood flow is reduced soon after the induction of diabetes in rats (Cameron *et al.*, 1991b), producing endoneurial hypoxia (Low *et al.*, 1987). In galactosaemic rats, nerve perfusion is also impaired (Myers & Powell, 1984; McManis *et al.*, 1986). Increased RICF under such conditions may be viewed as an adaptive response to improve ATP

supply by increased use of anaerobic metabolism (Low *et al.*, 1987). ARI treatment increases nerve blood flow (Yasuda *et al.*, 1989), which could be sufficient to restore ATP production and NCV. In addition, when polyol pathway flux is high, glucose is diverted through the pentose phosphate shunt to supply NADPH, a cofactor for aldose reductase (Dvornik, 1987); a process which requires ATP (Davidson & Murphy, 1985). Thus, polyol pathway activity may have both vascular and metabolic effects which contribute to RICF. When, however, these are corrected by ARIs there is no obvious adaptive stimulus to switch back to near total reliance on oxidative metabolism, given the glucose availability in diabetes. Thus, ARIs can restore NCV but, in a reversal experiment, simply halt the progressive increase in RICF.

The present results on RICF are in agreement with a previous study of partial prevention of the deficit by 25 mg kg^{-1} day^{-1} ponalrestat *in vivo* (Price *et al.*, 1988). In a recent report, however, Carrington *et al.* (1991) suggested that imirestat, a spiroimide-derived ARI, did not prevent the development of RICF, measured *in vitro*. We have replicated the present effect of ponalrestat (Cameron, Cotter & S. Hunter, unpublished observations), an acetic acid derivative, using a structurally unrelated sulphonylnitromethane compound (Mirrlees *et al.*, 1991). Spiroimide-derived ARIs stimulate Na-K ATPase independent of their effects on the polyol pathway (Garner & Spector, 1987), whereas ponalrestat does not. It is likely that such a chronic effect *in vivo* would cause increased ATP utilization, further encouraging the use of anaerobic metabolism, which would tend to increase RICF and cancel any beneficial effects of polyol pathway blockade. Between-study differences may also depend on diabetes duration, the experimental design, or the method of measurement. The investigation of Carrington *et al.* (1991) concerned preventative effects, over 1-month diabetes. Thus, that study examined the early stages of RICF development, whereas our investigation focused on later stages. In addition, Carrington *et al.* (1991) examined only the early stages of hypoxic conduction failure, evoked potentials being reduced by 20–40%, whereas this study examined reductions of $>80\%$ in all groups. Thus, the imirestat experiments monitored RICF in large myelinated fibres, which are most susceptible to diabetes (Cameron *et al.*, 1986b). In contrast, the present investigation examined all myelinated fibres; the differences between groups became more pronounced as evoked potential amplitude was reduced, indicating that smaller myelinated fibres benefit most from ARI treatment.

In conclusion, the data demonstrate polyol pathway involvement in the development of RICF in experimental diabetes. They also show that very high levels of polyol pathway blockade are necessary to normalize NCV. It is likely that ARI treatment of patients has been suboptimal and has not adequately tested the hypothesis that polyol pathway activity has an important role in the aetiology of diabetic neuropathy.

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