The aetiopathology of neuropathy in experimental diabetes

NORMAN E CAMERON

Abstract

Most information on the aetiology of experimental diabetic neuropathy comes from studies on rodent models, particularly the streptozotocin-diabetic rat. The major factor that impairs small and large nerve fibre function is a decrease in nerve and ganglion perfusion. This leads to reduced conduction velocity, increased resistance to ischaemic conduction failure, blunted regenerative capacity, painful neuropathy, and autonomic nerve dysfunction. Hyperglycaemia, altered lipid metabolism and reduced insulin action combine to cause adverse metabolic effects on vasa nervorum, vascular endothelium being a notable target. The resultant reduced vasodilation and increased vasoconstriction causes endoneurial hypoxia. Oxidative stress is of primary importance, due to increased production of reactive oxygen species from a plethora of intra- and extracellular sources. Advanced glycation and carbonyl stress play a supporting role, as does essential fatty acid dysmetabolism. These mechanisms are associated with alterations in cell signalling mediated by protein kinases, nuclear factor Kappa B and poly (ADP-ribose) polymerase.


Key words: neuropathy, vasa nervorum, blood flow, vasodilators, oxidative stress, advanced glycation, protein kinase, nuclear factor kappa B, poly (ADP-ribose) polymerase.

Introduction

Experimental diabetes causes major peripheral neurological changes similar to those seen in diabetic patients. These include early functional abnormalities, such as reduced sensory and motor nerve conduction velocity (NCV), resistance to ischaemic conduction failure, impaired nerve regeneration following injury, hyperalgesia, and autonomic changes such as reduced cardiac heart rate variability. In the first months of diabetes, these functional defects are not accompanied by gross morphological deficits of the kind seen in clinical neuropathy. Nonetheless, in the longer term, there is solid evidence for the development of damage to nerve and peripheral ganglion cell structure that resembles the situation in human disease.

Correspondence to: Professor Norman E Cameron
Department of Biomedical Sciences, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK.
Tel: +44 (0)1224 555713; Fax: +44 (0)1224 555719
E-mail: n.e.Cameron@abdn.ac.uk

Abbreviations

ACE = angiotensin-converting enzyme
AGEs = advanced glycation end-products
ARI = aldose reductase inhibitors
EDHF = endothelium-derived hyperpolarising factor
LDL = low density lipoprotein
MAPK = mitogen-activated protein kinase
NADH/NAD+ = reduced/oxidised nicotinamide-adenine dinucleotide
NADPH/NADP+ = reduced/oxidised nicotinamide-adenine dinucleotide phosphate
NCV = nerve conduction velocity
NFκB = nuclear factor kappa B
NO = nitric oxide
PARP = poly (ADP-ribose) polymerase
PKC = protein kinase C
RAGE = receptors for AGE
ROS = reactive oxygen species
SSAO = semicarbazide-sensitive amine oxidase
STZ = streptozotocin
lack of insulin production due to chemical destruction of a proportion of pancreatic beta cells. The animals are markedly hyperglycaemic but do not require insulin for maintenance. Other rodent models include BB/Wistar type 1 diabetic rats and more recently, Zucker type 2 diabetic rats. While the latter have been relatively understudied compared to the STZ-model, they all seem to have similar physiological and pharmacological profiles in terms of the aetiology of neuropathy. Most recently, attention is focussing on mouse models, with a view to genetic manipulation, however, while potentially exciting, little of significance has come from this approach to date.

Evidence from a large number of studies implicates impaired nerve and ganglion perfusion as the major aetiological factor in diabetic neuropathy. Indeed, this may be entirely responsible for early nerve dysfunction accounting, for example, for reduced sensory and motor NCV. In the longer term, vascular effects may be modulated by other events including direct effects of oxidative stress on neurons and Schwann cells and, in some experimental models, by changes in neurotrophic-related mechanisms.

Dysfunction of vasa nervorum vascular supply in diabetes and the pharmacology of vasodilator action

Diabetes has direct effects on rat vasa nervorum function, particularly for vascular endothelium, where deficits in the major vasodilator systems, nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarising factor (EDHF) have been noted. This also increases reactivity to vasoconstrictors such as noradrenaline, angiotensin II and endothelin-1; a phenomenon probably exacerbated by elevated local synthesis of angiotensin II and endothelin-1. The resultant pro-constricted state leads to an approximately 50% decrease in endoneural blood flow, coupled with a 30–40% reduction in oxygen tension. If this is the main cause of experimental neuropathy, then nerve function should be amenable to treatment with vasodilators, even though they do not necessarily correct the underlying defects in vasa nervorum, which are presumably of metabolic origin.

The major classes of conventional vasodilators correct nerve blood flow, NCV and abnormalities to varying degrees in diabetic rats (table 1). Most successful in restoring perfusion and function are those that block the renin-angiotensin system (angiotensin-converting enzyme (ACE) inhibitors and angiotensin II AT1-receptor antagonists), those that antagonise endothelin ETA receptors, and those that modulate vasa nervorum adrenoceptors (α1 antagonists and β2 agonists). Vasodilators generally do not alter the hyperglycaemia-dependent neurochemical changes in diabetes, including nerve polyol accumulation and diminished Na+, K+ ATPase activity, therefore these factors are only of minor importance in the aetiology of diabetic neuropathy. In addition to correcting NCV, vasodilators partially prevent the development of resistance to ischaemic conduction failure, a phenomenon that appears to depend primarily on increased nerve anaerobic metabolism coupled with reduced energy consumption by the Na+, K+ ATPase pump.

<table>
<thead>
<tr>
<th>Table 1. Vasodilator treatments that increase nerve conduction velocity and blood flow in experimental diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adrenergic system</strong></td>
</tr>
<tr>
<td>α1-adrenoceptor antagonists, β2-adrenoceptor agonists</td>
</tr>
<tr>
<td>e.g. prazosin, doxazosin, carvedilol, salbutamol</td>
</tr>
<tr>
<td><strong>Angiotensin system</strong></td>
</tr>
<tr>
<td>angiotensin-converting enzyme inhibitors and AT1-receptor antagonists</td>
</tr>
<tr>
<td>e.g. lisinopril, candesartan</td>
</tr>
<tr>
<td><strong>Calcium channels</strong></td>
</tr>
<tr>
<td>dihydropyridine type antagonists</td>
</tr>
<tr>
<td>e.g. nifedipine, nimodipine</td>
</tr>
<tr>
<td><strong>Endothelin system</strong></td>
</tr>
<tr>
<td>Endothelin ETA antagonants</td>
</tr>
<tr>
<td>e.g. BQ123, BMS182874</td>
</tr>
<tr>
<td><strong>Nitric oxide donors</strong></td>
</tr>
<tr>
<td>organic nitrates</td>
</tr>
<tr>
<td>e.g. isosorbide dinitrate</td>
</tr>
<tr>
<td><strong>Phosphodiesterase inhibitors</strong></td>
</tr>
<tr>
<td>e.g. cilostazol, pentoxyfylline</td>
</tr>
<tr>
<td><strong>Potassium channels</strong></td>
</tr>
<tr>
<td>ATP-sensitive potassium channel openers</td>
</tr>
<tr>
<td>e.g. celikalim</td>
</tr>
<tr>
<td><strong>Serotonergic system</strong></td>
</tr>
<tr>
<td>5HT2 antagonists</td>
</tr>
<tr>
<td>e.g. ritanserin, sarpropelate</td>
</tr>
</tbody>
</table>

Vasodilators such as the ACE inhibitor, lisinopril, also ameliorate defects in autonomic ganglia blood flow and small nerve fibre dysfunction. In the latter case lisinopril prevented the development of impaired responses of the gastric fundus to stimulation of the nitrergic nerve fibres that normally relax this tissue. Lisinopril treatment also reduced some measures of painful neuropathy, including thermal hyperalgesia but had no effect on pressure pain thresholds.

Diabetes causes nerve fibre degeneration and blunted regenerative capacity in experimental models and patients. In diabetic rats, the regeneration defect following nerve damage was corrected by vasodilator treatment with an angiotensin AT1-receptor antagonist, indicating that a reduced energy supply severely limits nerve regrowth.

Thus, vasodilator treatment alone can correct the major early defects in nerve function in experimental diabetes. This has parallels for human studies; limited clinical trials using ACE inhibitors have revealed some improvements in symptoms and objective measures such as NCV, that at least match the benefits found for inhibitors of metabolic targets such as aldose reductase or protein kinase C (PKC). Given that many of the metabolic alterations in diabetes that are discussed below have vascular consequences, the question that arises is to what extent the beneficial actions of inhibitors of dysmetabolism derive from their effects on nerve perfusion, as opposed to a direct action on neurons and Schwann cells themselves?

Our group has a substantial database for therapeutic intervention in the STZ-diabetic rat model under standardised condi-
tions, which permits an answer to this question.\textsuperscript{1,13} In figure 1, these data have been plotted to show the relationship between sciatic motor NCV and endoneurial blood flow. The data are divided into different treatment categories: vasodilators, antioxidants, aldose reductase inhibitors (ARI), PKC inhibitors, and other miscellaneous treatments (including cannabinoids, essential fatty acids, aminoguanidine, myo-inositol, statins, poly [ADP-ribose] polymerase and nuclear factor kappa B [NFκB] inhibitors, C-peptide, and drug mixtures, for example lipic acid – gamma-linolenic acid. All the curves are similar and fit the data well: conduction velocity is low at low blood flow rates and reaches an asymptote at high flows, corresponding to the non-diabetic level. This emphasises the importance of blood flow changes to nerve conduction, regardless of the type of drug intervention.

left would have been expected if there were important non-vascular/neural actions on top of the vascular effects. Thus, the evidence is overwhelmingly in favour of a predominant vascular modus operandi of these ‘metabolic’ drugs. It remains to determine precisely how such metabolic factors affect nerve perfusion.

Metabolic factors responsible for impaired nerve perfusion and function

There are numerous metabolic changes in diabetes of relevance for neuropathy, many but not all of which are ultimately driven by hyperglycaemia and/or hyperlipidaemia. Thus, the polyol pathway is activated; non-enzymatic glycation and the formation of advanced glycation end-products (AGEs) is increased; reactive oxygen species (ROS) production is elevated and antioxidant defences may be compromised resulting in a state of oxidative stress; lipid metabolism is altered. These processes, to a degree, interact and are interdependent, together activating cascades of downstream events in cell signalling, alterations in phenotypic expression, and cell death pathways towards necrosis and apoptosis. Important intermediary players emerging in the context of neuropathy include kinases such as PKC, NFκB, and PARP.

Oxidative and nitrosative stress

In the past 10 years, research using animal models has identified oxidative stress as perhaps the most important single mechanism underlying the aetiology of diabetic neuropathy and vasculopathy. ROS formation is increased by diabetes (figure 2); potential sources are numerous and are both intra- and extracellular.
Leakage of superoxide from the mitochondrial respiratory chain is considered a major intracellular ROS source. Many intracellular metabolic reactions also produce superoxide and hydrogen peroxide, including cytochrome P450, phospholipase A, and NAD(P)H oxidase. The latter enzyme is important in vascular cells, and is upregulated in hypertension, which is a risk factor for neuropathy. NO synthase may also produce superoxide, at least under pathophysiological conditions when the supply of substrate (L-arginine) or the co-factor tetrahydrobiopterin is restricted.

ROS may also result from the blood flow problems they cause in diabetes, during ischaemia-reperfusion episodes, with a contribution from the xanthine oxidase system. Moreover, diabetes increases hepatic release of xanthine oxidase into the circulation, binding to vascular endothelium to potentially exacerbate the situation. Glucose autoxidation and the advanced glycation process are other ROS sources elevated by diabetes. Levels of semicarbazide-sensitive amine oxidase (SSAO) are increased in diabetic rats and patients. This enzyme is found in several tissues, with a high concentration in vascular smooth muscle, and metabolises substrates such as methylamine and aminoacetone, which are elevated by diabetes, to produce hydrogen peroxide. Peroxisomes are another important source of hydrogen peroxide, particularly from beta-oxidation of medium chain fatty acids. Low density lipoprotein (LDL) is both oxidised and glycated in diabetes, and may, therefore, also contribute to nerve vascular dysfunction, being a potential source of ROS transfer to endothelium.

There is also the possibility of nitrosative stress, defined as an excess of reactive nitrogen species such as N$_2$O$_3$, NO, and particularly in the context of diabetes, peroxynitrite (figure 2). The latter is formed from the interaction of superoxide and NO. Peroxynitrite can decompose spontaneously to the most extremely cytotoxic of ROS, the hydroxyl radical. Peroxynitrite, also nitrates and nitrosylates proteins, potentially altering their function. Increased concentrations of the nitration product, nitrotyrosine, have been observed in vascular tissues from diabetic patients and animals. Nitrosative stress could be driven by excess production of NO, for example during inflammatory processes after stimulation of inducible NO synthase. However, in experimental diabetes, it is likely that the driving force is increased superoxide production in a setting of relatively normal NO synthesis.

Thus, there are many putative mechanisms for ROS formation in diabetes, although any systematic attempts to dissect the relative importance of the individual pathways have yet to be published. Much of the evidence for the importance of ROS in experimental diabetic neuropathy comes indirectly from studies using general free radical scavengers such as butylated hydroxytoluene, probucol, vitamin E, beta-carotene and alpha-lipoic acid. These prevent or correct most early nerve dysfunction, including NCV, nerve blood flow and oxygenation deficits, and impaired nerve fibre regeneration following damage. Scavengers also improve ganglion blood flow and small nerve fibre function, including responses of the nitrergic innervation of corpus cavernosum and mechanical and thermal hyperalgesia. NCV and nerve blood flow deficits may be mimicked in non-diabetic rats by chronic treatment with the pro-oxidant, primaquine, in the absence of hyperglycaemia. This stresses the importance of glucose/lipid-induced ROS production for neurovascular dysfunction in diabetes. Elevated superoxide formation by epi-neurial vessels from diabetic rats is reduced by antioxidant treatment. Superoxide is dismutated to hydrogen peroxide; the latter is then converted to hydroxyl radical like species by Fenton chemistry, catalysed by free transition metal ions such as iron or copper. The importance of the hydroxyl radical for nerve and vascular dysfunction has been stressed by employing more specific scavengers such as dimethylthiourea.

A major disadvantage of the scavenger approach, which may limit its appeal to proof of concept rather than therapeutic potential, is that very high concentrations must be present in tissues to intercept ROS whenever they are formed, in competition with the cellular ROS targets. An alternative approach is to focus on the mechanisms of ROS synthesis, or transformation to hydroxyl radical. In this context, transition metal catalysed reactions are interesting, because they are involved in ROS formation by glucose autoxidation and advanced glycation, as well as in hydroxyl radical synthesis from hydrogen peroxide. Experimental diabetes is associated with transition metal dysregulation rather than gross overload, therefore, treatment with relatively low doses of metal chelators may be appropriate. The data support this notion; chelator treatment with deferoxamine or trientine had marked beneficial effects on NCV, nerve blood flow, corpus cavernosum neural and endothelial dysfunction, and vasodilation dependent on endothelial NO and EDHF systems. Furthermore, a single dose of chelator was effective for 2–3 weeks, presumably because it takes that time to build up levels of free transition metals once they have been depleted by chelation and excretion.

**AGE product formation and carbonyl stress**

It is difficult to disentangle the functional effects of oxidative stress and AGE formation because the latter process produces ROS and is accelerated by them. Indeed, antioxidant treatment can reduce tissue AGE levels. Figure 3 illustrates the early processes involved in advanced glycation, highlighting the conversion of glucose to AGEs via intermediates of Amadori product breakdown such as the dicarbonyl, 3-deoxyglucose. Other dicarbonyl products of glucose metabolism, such as glyoxal and methylglyoxal also make an important contribution to AGE formation. As the process depends on these increases in reactive carbonyl compounds, diabetes has been considered as a state of carbonyl stress. In addition to acting as a source of ROS, AGEs can quench NO. AGE formation promotes protein crosslinking and can degrade the function of important enzymes, including NO synthase. Agents such as aminoguanidine bind to dicarbonyl species, thus preventing further AGE-producing reactions. Aminoguanidine improves NO-mediated endothelium-dependent vasodilation, NCV and nerve blood flow in diabetic rats.
However, it is not entirely clear that aminoguanidine’s anti-AGE action is solely responsible for these beneficial neurovascular effects. Thus, aminoguanidine is also a potent inhibitor of SSAO and could therefore reduce ROS formation by this mechanism. Moreover, the build up of AGEs seen in nerve and vascular tissue in vivo is a slow process, taking considerably longer after diabetes induction than the development of NCV and nerve perfusion deficits. Another anti-glycation agent, OPB-9165, partially prevented sciatic nerve AGEs accumulation and motor NCV deficits after prolonged (six month) experimental diabetes. However, the precise mode of action of OPB-9165 is uncertain because it also attenuated sciatic nerve oxidative stress-related DNA damage and could, therefore, have been functioning as an antioxidant.

A further possibility is that rapidly formed circulating AGEs (including those from dietary sources) could be the potential trigger for neurovascular alterations, acting to stimulate endothelial receptors for AGE (RAGE) receptors. The latter are involved in activation of transcription factors such as NFκB; ROS are involved in cell signalling for this pathway, which may account for similarities in the neurovascular effects of antioxidants and AGE inhibitors in diabetic rats.

### Polyol pathway activation

The polyol pathway (figure 4) is present in many tissues including peripheral nerve and blood vessels. Glucose is converted to sorbitol by aldose reductase, and sorbitol may be further metabolised to fructose, catalysed by sorbitol dehydrogenase. Flux through this pathway alters cell redox state in terms of reduced/oxidised nicotinamide adenine dinucleotide phosphate (NADPH/NADP+) and reduced/oxidised nicotinamide adenine dinucleotide (NADH/NAD+) ratios. In contrast to the modest beneficial effect of ARIs in clinical trials, they are highly effective in animal models. At least part of the reason for this discrepancy is the doses employed in clinical trials, which have been very much lower than in pre-clinical studies. In fact, the levels of pathway inhibition attained in trials would not be sufficient to correct nerve dysfunction in diabetic rats. The accumulation of sorbitol and fructose in nerves of diabetic rats is irrelevant to the development of nerve dysfunction. This is clear from studies employing vasodilators, antioxidants or a variety of other therapeutic approaches which restore nerve function without affecting polyol accumulation. Indeed, therapeutically, the nerve metabolite levels are likely to be an epiphenomenon, the most likely target of ARI action being vasa nervorum.

The vascular action of ARIs and the clear relation with amelioration of NCV deficits in diabetic rats (figure 1) primarily involves correction deficits in NO- and EDHF-mediated endothelium dependent vasodilation. This could theoretically depend on redox changes and an NADPH-sparing effect of ARIs because NO synthase requires NADPH, resulting in competition with aldose reductase. However, there is no direct evidence for this view. In vascular and neural tissues of diabetic rats, ARI treatment corrects a deficit in synthesis of the endogenous antioxidant, glutathione. Thus, ARIs function indirectly to reduce oxidative stress.

A further possibility is that rapidly formed circulating AGEs (including those from dietary sources) could be the potential trigger for neurovascular alterations, acting to stimulate endothelial receptors for AGE (RAGE) receptors. The latter are involved in activation of transcription factors such as NFκB; ROS are involved in cell signalling for this pathway, which may account for similarities in the neurovascular effects of antioxidants and AGE inhibitors in diabetic rats.
Lipid-metabolism related effects
Diabetic rats show hypertriglyceridaemia and hypercholesterolaemia, which could contribute to oxidative stress and atherogenesis. Diabetes also impairs the metabolism of essential fatty acids.

Thus, hepatic Δ-6 and Δ-5 desaturation of essential fatty acids is suppressed by diabetes, due to reduced insulin action and perhaps oxidative stress. This limits conversion of the dietary ω-6 component, linoleic acid, to precursors that are further metabolised to 1 and 2 series prostanoids and lipoxigenase products. Several of these metabolites are vasoactive, including the powerful vasodilator and inhibitor of platelet aggregation, prostacyclin. The rate limiting powerful vasodilator and inhibitor of platelet aggregation, prostacyclin. The rate limiting

The hypercholesterolaemia in diabetes prompted recent studies using statins to block HMG-CoA reductase and the cholesterol biosynthesis pathway. While this had no effect whatsoever on circulating cholesterol levels in diabetic rats and mice, the experiments nevertheless revealed pleiotropic beneficial effects of statin treatment. These included improved vascular NO-mediated endothelium-dependent relaxation, NCV, nerve blood flow and corpus cavernosum nitric nerve function (NE Cameron, MA Cotter, MR Nangle, unpublished observations 2002). While the results did not depend on plasma cholesterol lowering, they did depend upon inhibition of the cholesterol biosynthetic pathway because co-treatment with a high dose of the HMG-CoA reductase metabolite, mevalonate, abolished statin effects.

Downstream mechanisms
Vascular PKC activity is elevated by diabetes, particularly for the beta isoform. One potential stimulus for PKC activation is increased de novo synthesis of diacylglycerol from glucose. However, both PKC activity and diacylglycerol mobilisation from lipids are elevated by the metabolic processes causing oxidative stress in diabetes, and this is probably the most important in vivo stimulus. Thus, in terms of aetiology, the PKC mechanism lies downstream of ROS and AGE formation, and polyol pathway stimulation, and may in part mediate their adverse neurovascular effects. For example, PKC-mediated phosphorylation of endothelial NO synthase reduces NO-mediated vasodilatation. A wide range of beneficial effects have been found in diabetic rats using both isoform non-specific and β-isofrom-specific PKC inhibitors, for both motor and sensory NCV, thermal pain thresholds, nerve and ganglion blood flow, and endothelium-dependent vasodilatation mediated by the NO and EDHF systems.

The mitogen-activated protein kinases (MAPKs) constitute another collection of downstream cell signalling pathways currently under investigation by several groups. They may be activated by cellular stress (for example by hyperglycaemia or ROS) in both neurons and vascular cells. For the former, this has been shown for cultured rat sensory neurones and dorsal root ganglion cells in vivo. In diabetic rats, p38 MAPK inhibition partially prevented reduced sensory NCV.

The nuclear enzyme, PARP, is involved in the DNA repair process and is stimulated by DNA strand breakage, caused, for example, by ROS. When strongly activated, the formation of poly (ADP-ribose) produces a severe energy stress on cells, depleting ATP and NAD+ levels to an extent sufficient to limit oxidative metabolism and perhaps drive cells towards necrotic death. Ongoing PARP activation has been implicated in impaired vascular endothelium NO-dependent vasodilatation in diabetes, which can rapidly be restored by PARP inhibitors. Recently this vascular effect has been shown to contribute to nerve dysfunction in diabetic rats. Thus, PARP inhibition corrects NCV and blood flow defects in diabetic rats, and preliminary data show correction of impaired gastric fundus nitric innervation responses (TM Gibson, MA Cotter and NE Cameron, unpublished observations 2002). It is interesting to note that PARP also has other functions, including a role in the regulation of chemokine production and inflammatory responses, as well as in the control of NFκB expression. The latter may be important even when PARP activation is at a relatively low level in diabetes, not sufficient to cause severe cellular energy stress or cell death. In that case PARP would nonetheless exacerbate deleterious ROS and AGE effects on neurons and their vascular supply.

Trophic effects
While it is clear that nerve blood flow deficits play a major role in the aetiology of experimental diabetic neuropathy, there have also been numerous reports of defects in trophic or neurotrophic factors or mechanisms. In some reports, treatment with an appropriate trophic agent prevented or corrected aspects of nerve dysfunction such as NCV or pain thresholds in diabetic rats. These agents include insulin-like growth factor, ciliary neurotrophic factor, brain-derived neurotrophic factor, neurotrophin-3, vascular endothelium growth factor, nerve growth factor (small fibre effects only) and prosaposines. In the majority of studies it is not clear what causes the neurotrophic defects in the first place, and the precise mode of action of the trophic agents is not established. It is plausible that oxidative stress or some other factor such as impaired target tissue blood flow limits availability of trophins in diabetes. Some of the trophic agents, such as insulin-like growth factor, ciliary neurotrophic factor, and vascular endothelium growth factor have direct vascular actions and may act via improvements in nerve blood flow. However, the other agents listed do not have documented neurovascular effects. One limitation with the research is that experiments were carried out with diabetes induction in relatively young rats, and a proportion of their NCV deficits will be due to blunted nerve fibre growth and maturation. It could be that restoring this growth is the main target of these trophic drugs, although the relevance to human neuropathy, which develops when nerves are mature, is unclear. However, if growth results in an increase in nerve metabolic rate, then elevated blood flow would be expected to follow, as happens during the normal maturation process, after nerve damage, and when diabetic nerves are subjected to repetitive electrical activation.
**Synopsis, conclusions and interactions between mechanisms**

Experimental diabetic neuropathy clearly has a multifactorial aetiology, schematised in figure 5. Vascular dysfunction is the prominent result of multiple metabolic insults, the vascular endothelium being a major target. There are diabetic deficits in the major endothelium-derived vasodilators, NO, EDHF and prostacyclin, as well as enhanced vasoconstriction due to angiotensin II and endothelin-1. Normally, these mechanisms interact in an integrated system for nerve blood flow control, however, diabetes shifts this towards ischaemia and endoneurial hypoxia, which causes nerve dysfunction and eventual neurodegeneration. Oxidative and carbonyl stress and their inter-related mechanisms result from hyperglycaemia and dyslipidaemia, activating a number of downstream signalling pathways such as protein kinases, PARP and NfkB to cause neurovascular dysfunction.

Given the complexity of this aetiologic scheme and the possibility of numerous sites for therapeutic intervention at different levels, it is likely that multiple drug therapy could prove advantageous, as in some other cardiovascular diseases such as hypertension. Experimental approaches in animal models have revealed several instances of synergistic drug interactions. These include antioxidant and ω-6 essential fatty acid combinations, ACE inhibitors and ARIs; ACE inhibitors or angiotensin AT1 receptor antagonists and statins; AT1- and endothelin ETA-receptor antagonists; and antioxidants or ω-6 essential fatty acids with PKCβ inhibitors.1,11,21 Low doses of several of these agents (ARIs, ACE and PKCβ inhibitors, ω-6 essential fatty acids, antioxidants) singly have already been shown in clinical trials to have some beneficial effects on human diabetic neuropathy. Animal experiments would, therefore, predict that use of several drug combinations could give a marked therapeutic advantage.

**Acknowledgements**

The writing of this review was supported in part by a grant from the MRC. I am grateful to Professor Mary A Cotter for discussion and critical comment.

**References**

9. Low PA, Nickander KK. Oxygen free radical effects in sciatic nerve in