SUMMARY - This review orders the likely components of the pathogenesis of diabetic neuropathy into vertical (temporal) and horizontal dimensions. It is argued that the effects of hyperglycaemia are transduced to neuronal dysfunction via at least three secondary biochemical disturbances - the sorbitol (polyol) pathway, non-enzymatic glycation of proteins and oxidative stress - and that there are clear interactions between them. Because of these interactions, interference with one of these biochemical transducers could either worsen or attenuate the effects of the others. Examples of these alternatives are given. It is suggested that the prime goal for pharmacological intervention should be a combined attack on all three sources of disturbance. Interventions further on in the sequence of pathogenesis are also considered, and the arguments for the use of neurotrophic factors are persuasive because of their selectivity for different neuronal phenotypes, even though side-effects may be inevitable. Finally, a novel conjugate of γ-linolenic acid and α-lipoic acid is considered as an agent with the potential to correct effects arising from more than one pathway of disorder in experimental diabetic neuropathy. The preliminary results with this agent have been encouraging. Diabetes & Metabolism 1998, 24, 79-83.

Key-words: nerve growth factor, neuropathy, neurotrophin-3, neurotrophins, non-enzymatic glycation, oxidative stress, polyol pathway.

RÉSUMÉ - La prévention et le traitement futurs de la néphropathie diabétique. Cette revue ordonne les composants probables intervenant dans la pathogénie de la neuropathie diabétique dans les dimensions verticale (temporelle) et horizontale. Il est suggéré que les effets de l'hyperglycémie conduisent à une dysfonction neuronale par l'intermédiaire d'au moins trois anomalies biochimiques secondaires (la voie du sorbitol (polyol), la glycation des protéines et le stress oxydatif) et qu'il y a de claires interactions entre elles. Du fait de ces interactions, l'interférence avec un de ces effecteurs biochimiques pourrait empirer ou atténuer les effets des autres. Des exemples de ces modulations sont donnés. Il a été suggéré que l'objectif essentiel d'une intervention pharmacologique devrait être une attaque combinée de ces trois sources d'anomalies. Des interventions plus tardives dans la séquence de la pathogénie ont également été considérées, et l'utilisation des facteurs neurotrophiques s'est révélée efficace grâce à leur sélectivité pour les différents phénomènes neuronaux, même si des effets secondaires sont souvent inévitables. Enfin, un nouveau composé conjugué d'acide γ-linoléénique et d'acide α-lipoïque semble avoir le potentiel de corriger les anomalies de la neuropathie diabétique expérimentale résultant de plus d'un de ces effecteurs biochimiques. Les résultats préliminaires obtenus avec cet agent sont prometteurs. Diabetes & Metabolism 1998, 24, 79-83.

Mots-clés : facteur de croissance nerveux, facteurs neurotrophiques, glycation des protéines, neuropathie, neurotrophin-3, voie du polyol, stress oxydatif.

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It is now clear from the Diabetes Control and Complications Trial [1, 2] that the pathogenesis of diabetic neuropathy derives from some manifestation of poor control, though this was already a strong probability from the founding studies of Pirart [3]. It is generally assumed that the causative element from poor control is hyperglycaemia, and this lends focus to a second level of biochemical disturbances. The principal candidates are the polyol pathway and non-enzymatic glycation of proteins, though undiscovered alternatives are possible (Fig. 1). These have clear connections with oxidative stress, in that both exaggerated flux through the polyol pathway and the formation of glycated protein adducts can increase the generation of free radicals as well as impair their scavenging [4-10]. Thus, this group of secondary biochemical transducers form related drug targets, typified by aldose reductase inhibitors and antioxidants such as α-lipoic acid which might be mutually-supportive when given in combination. We await a useful inhibitor of protein glycation, and the development of such a drug will provide an enormous leap forward in both understanding and therapeutic potential. The potential importance of protein glycation in the development of diabetes complications can only be guessed at without the pharmacological means to inhibit the process. Furthermore, it is impossible to properly judge the usefulness of agents acting on the other processes (i.e. aldose reductase or oxidative stress) whilst the glycation process remains intact. For example, it is logical that inhibition of aldose reductase might increase the concentration of glucose at a site of protein glycation. It is possible that such an increase in glycation might offset the benefits accrued from an aldose reductase inhibitor, and this may explain in part their poor clinical performance. Thus, the availability of potent specific inhibitors of non-enzymatic protein glycation will increase the potential of aldose reductase inhibitors and antioxidants.

As a general principle, pharmacological intervention in a pathogenetic sequence of events should be made as early in the pathway as possible. Thus, the best attack on diabetic neuropathy is prevention of hyperglycaemia at the source. We all know this is not possible in most patients for much of the time, and this fact, taken together with the principle at the top of this paragraph, vindicates the need to intervene at the next stage. Therefore, the development of successful drugs targeted at oxidative stress, the polyol pathway and protein glycation must represent the best goal at this stage in our understanding of the problem. Such drugs would have the added advantage of potential against the other complications of diabetes.

At some stage, these second-level biochemical abnormalities must translate to dysfunction, and the transducers for this stage are interesting and mysterious. It is possible that the earliest functional manifestations are conduction disturbances. These are clearly related to the polyol pathway in diabetic rats [11], but this relationship is not well-defined in man (Fig. 2). It is further argued that reduced blood flow in nerve trunks acts as an intermediate dysfunctional step en route to decreased conduction (Fig. 1), but this may be questionable for at least three reasons. It is difficult to see how nerve trunk ischaemia could precipitate a neuropathy that is usually predominantly sensory, and autonomic dysfunction can develop in diffuse nerve plexi where there is no indication of local ischaemia. Secondly, Mendenhall’s syndrome (a profound, almost untreatable hyperglycaemia resulting from a genetic mutation of the extracellular domain of the insulin receptor) is associated with severe neuropathy without any abnormalities of endoneurial microvessels [12].

**Fig. 1.** Schematic to indicate the vertical (temporal) and horizontal dimensions of the pathogenesis of diabetic neuropathies. Destructive effects of hyperglycaemia are transduced by a range of secondary biochemical defects, with the polyol pathway, oxidative stress and protein glycation clearly identified and clearly linked. The origins and sequence of functional defects are less clearly defined. Question marks indicate the probability of other factors as yet unidentified.

**Fig. 2.** The natural history of diabetic neuropathy in rats and patients. Changes in severely diabetic (streptozotocin) rats are clearly related to hyperglycaemia and are relatively acute; they parallel early-onset changes in patients with badly controlled diabetes. These are best termed hyperglycaemic neuropathy. True diabetic streptozotocin has a quite different profile.
Thirdly, most of the reduced nerve blood flow in diabetic rats derives from muscle wasting rather than from a biochemical abnormality related to diabetes; thus non-diabetic control rats that have been under-fed to cause muscle wasting also show a profound reduction in sciatic nerve blood flow [13]. Indeed, sciatic nerve blood flow correlates well with the combined weight of hindlimb muscles. This observation is clearly explained elsewhere [13], and it is argued that it invalidates most of the work on nerve blood flow in diabetic rats. There is, however, good evidence for clinical manifestations of endoneurial ischaemia [14-16], but all of this evidence comes from the late stages of the disease, indicating that impaired nerve blood flow probably parallels these late stages. Thus, nerve ischaemia probably participates late in pathogenesis; its pharmacological correction may not influence a neuropathy which may have become irreversible, so that neurovascular changes are not a good drug target.

The neurotrophic factors, and particularly the superfamily called the neurotrophins, offer an alternative pathogenetic mechanism that can give rise to distal neuropathy affecting selective neurone groups. This is because individual neurotrophins modulate the expression of phenotype in different classes of neurone via selective high-affinity receptors (Fig. 3). Deficits of expression and function of nerve growth factor (NGF) in diabetes have been studied extensively and are summarised for the rat model in Figure 4. In essence, the NGF deficit in experimental diabetes has two components: impaired expression of NGF in its target tissue sources (skeletal muscle and skin) and impaired expression of one or more of the two types of receptor that capture NGF and bring it into the neurone. Impaired expression is clearly demonstrable in diabetic rats in soleus muscle and foot skin, both as deficits in mRNA and in NGF-like immunoreactivity [17-19]. Defective NGF receptor function in diabetic rats has been suspected for many years because early experiments showed reduced capture and retrograde transport of exogenous labelled NGF [20, 21]. More recently, it has been shown that the deficit is primarily reduced neuronal expression of p75NTR with normal levels of trkA, and there is an excellent correlation between the reduced ganglionic levels of p75NTR and retrograde axonal transport of endogenous NGF [22].

The deficit in retrograde transport of NGF is clearly associated with reduced expression of two of its neuronal target genes: those coding for substance P (via the precursor peptide, preprotachykinin A) and for calcitonin gene-related peptide (CGRP). Administration of exogenous NGF corrects this [17, 23]. These effects of NGF on substance P expression in diabetic rats extend to changes in release of transmitter [24] and nociception [25]. Thus, from both biochemical and functional standpoints, animal studies implicate impaired neurotrophic support from NGF in C-fibre dysfunction in diabetes and indicate a role for exogenous NGF in therapy. A single clinical study indicates that reduced levels of NGF are also present in the skin of a small sample of diabetic patients [26].
These studies give a sound basis for the clinical trials on NGF that are on-going [27].

In spite of optimism for NGF, it is clear that C-fibres play a modest role in the range of protective sensation in the foot, and it is the loss of this that predisposes the diabetic neuropathic patient to ulceration and amputation. Clearly we must attend to other fibre types that contribute to protective sensation, and this draws interest to large myelinated sensory fibres which express trkC receptors and whose phenotype is probably maintained by neurotrophin 3 (NT-3). Our ability to explore the influence of NT-3, by following the lines of investigation laid out by the NGF studies referred to above, has been limited by lack of an effective assay for NT-3 and no knowledge of its neuronal gene targets. The former has been rectified, and we know that, as with NGF, diabetic rats show reduced axonal transport of NT-3 in the sciatic nerve [28]. Furthermore, administration of human recombinant NT-3 prevents the development of reduced conduction velocity in large-diameter (fast-conducting) sensory fibres of the sciatic nerve in diabetic rats [19]. We do not understand the mechanism of this effect on conduction velocity, but on pragmatic grounds it offers some promise for therapeutic effects of NT-3 in diabetic neuropathies.

Although treatment with neurotrophins offers selective manipulation of neuronal phenotype, it requires injection of an exogenous protein which may have effects on neurones that are not dysfunctional in diabetic patients. Although injection of exogenous protein might be better tolerated in diabetic patients accustomed to insulin administration than in those suffering from many other diseases, it would be a novel experience for most Type 2 patients and is not the most desirable means of delivery of therapeutic agents. There is also the question of side-effects, and this problem is typified by the local hyperalgesia and allodynia caused by the relatively high concentrations of NGF at the injection site [29]. Consequently, the reduction of tissue levels of NGF in diabetes may also serve as a useful end-point for the assessment of forms of therapy that may not suffer from undesirable side-effects. Furthermore, it was emphasised earlier in this account that interventions at a fundamental stage in pathogenesis are preferable to those made later. Thus, correction of hyperglycaemia, if possible, would be preferable to administration of recombinant neurotrophins. As stated above, euglycaemia in diabetic patients is not realistic, but new developments in the field of antioxidant therapy may strike very close to the fundamental problems of disordered biochemistry. The mitochondrial antioxidant, \( \alpha \)-lipoic acid (thioctic acid), has been used in Germany for some years now with solid claims of efficacy against conduction and sensation disorders and autonomic neuropathy in diabetic patients [30-32]. We have shown that in diabetic rats it can increase sciatic nerve levels of NGF and stimulate expression of its neuronal target, substance P [24]. More recently, studies with a new conjugate of \( \gamma \)-linolenic acid and \( \alpha \)-lipoic acid (GLA-LA) have indicated increased potency. This agent has the capacity to correct both conduction and neurochemical defects – with positive effects on NGF and substance P together with a stimulation of sciatic nerve levels of neuropeptide Y (Fig. 5). This latter observation is of great importance, though its functional significance is not yet clear. The studies referred to above show that a reversal of the substance P depletion in sciatic nerves of diabetic rats is also seen with exogenous NGF treatment [17, 24], and, in the pattern of changes shown in Figure 5, substance P follows the changes in endogenous NGF. However, NGF administration does not affect the deficit in sciatic nerve neuropeptide Y [24], so that its correction by the GLA-LA conjugate implies that this agent has beneficial effects on neurotrophic mechanisms over and above those on NGF. Thus, we may have a novel therapeutic agent that can prevent a wider range of neurological defects in diabetics than those tested so far, incorporating as yet...
unidentified consequences of oxidative stress. This promises well for the future.

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