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Review

Mitogen-activated protein kinases as glucose transducers for diabetic complications

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Abstract

The damaging effects of glucose on the cells which contribute to the development of diabetic complications are ill-understood. There are three major hypotheses – the sorbitol pathway, non-enzymatic glycation of proteins and increased oxidative stress – and many examples illustrate inter-connections between the three. It is suggested that these pathways, together with other biochemical anomalies arising from hyperglycaemia, can synergise by sharing the capacity to activate mitogen-activated protein kinases (MAP kinases) and that these enzymes in actual fact form glucose transducers. The more recent hypothesis, namely that activation of a specific isoform of protein kinase C (PKC) underpin damaging changes in retinopathy and neuropathy, can also be related because protein kinase C is an effective activator of mitogen-activated protein kinases. These latter kinases phosphorylate transcription factors, which in turn alter the balance of gene expression. In this way they can alter cellular phenotype, promote division or increase production of extracellular material. In short, mitogen-activated protein kinases have the capacity to trigger all the cellular events necessary for the development of diabetic nephropathy, retinopathy and neuropathy and it is suggested that their pharmacological modulation might provide therapeutic control of these conditions. [Diabetologia (1999) 42: 1271–1281]

Keywords Nephropathy, neuropathy, retinopathy, hyperglycaemia, MAP kinase, ERK, JNK, p38, protein kinase C, aldose reductase.

The results of the Diabetes Control and Complications Trial [1] emphatically confirmed the heroic studies of Jean Pirart [2–4], showing that poor control of glycaemia in Type I (insulin-dependent) diabetes mellitus predisposes patients to chronic complications. This has led to the tacit assumption that glucose, at supranormal concentrations, is the agent of damage. Participation of hypoinsulinaemia or other manifestations of poor control cannot be discounted but glucose itself certainly has the capacity to disturb biosystems. It can do this in a variety of ways, so that hypotheses to explain specific complications – retinopathy, nephropathy and neuropathy – starting from high glucose are tenable, testable and, therefore, useful.

Figure 1 presents a simple generic plan by which complications develop. It usefully discriminates between metabolic effects of glucose on the target cells showing the complication and the exacerbating effect of independent accelerators, such as arterial hypertension in diabetic nephropathy. Not all independent accelerators have, however, as clearly defined a rela-

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Abbreviations: ERK, Extracellular signal regulated kinase; JNK, c-jun N-terminal protein kinase; GLA-LA, diester of gamma linolenic acid and alpha-lipoic acid; GSH, reduced glutathione; MAP kinase, mitogen-activated protein kinase; NGF, nerve growth factor; PKC, protein kinase C; RAGE, receptors for advanced glycation end-poducts; VEGF, vascular endothelial growth factor.

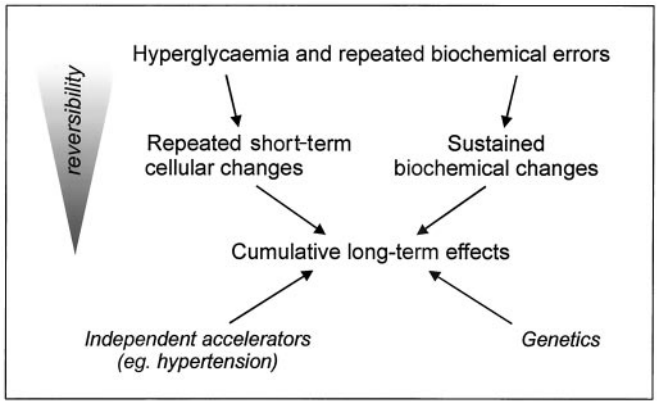

Fig. 1. General schematic to indicate pathways for the development of diabetic complications (after Giancarlo Viberti, personal communication). Cellular transducers register the effects of hyperglycaemia and related extracellular biochemical anomalies and convert them to cumulative long-term effects. As short-term biochemical changes translate into long-term effects, with structural anomalies, reversibility is progressively lessened. Independent accelerators (such as arterial hypertension in nephropathy) and genetic predisposition amplify these biochemical effects.

tion with the primary cause of a complication and the nature of this subtlety is important in the design of new drugs aimed at preventing or treating the complication. This will be considered in more detail later.

Explaining the effects of glucose requires identification of the mechanisms by which it disrupts cellular metabolism. A few hypotheses have emerged to explain the initial steps in the effects of glucose. The most clearly defined are the sorbitol hypothesis [5–7] and non-enzymatic glycation of proteins [8–10]. These detailed sources indicate that both metabolic anomalies can contribute to the development of diabetic complications and that they overlap with each other and with other diabetic anomalies. For example, oxidative stress clearly interacts with both the sorbitol pathway [11–13] and non-enzymatic glycation [14–16]. Indeed, it is clear that oxidation of glucose opens up alternative pathways for protein glycation and could, therefore, increase its range and extent, with an amplification of the consequences [14, 17–19]. It is, therefore, important to establish which components of superficially separate metabolic anomalies interact and synergise and by what mechanisms. The literature on the various phenomena which stem from hyperglycaemia, osmotic stress, oxidative stress, glycation and so forth, reveals a recurring topic, namely mitogen-activated protein kinases.

Mitogen-activated protein (MAP) kinases

Having introduced the potential involvement of MAP kinases in responses to diabetes-derived cellular changes, it is necessary to consider their biochemistry, classification and nomenclature. Mitogen-activated protein kinases form a group of serine/threonine specific kinases which are activated in response to extracellular stimuli through dual phosphorylation at conserved threonine and tyrosine residues. There are three main groups of MAP kinases; the extracellular signal regulated kinases (ERK), the c-Jun N-terminal kinases (JNK) and the p38 kinases. Although ERK, JNK and p38 are frequently referred to as single entities, the terms refer to homologous groups of kinases with similar activation profiles and substrates. Thus, ERK and JNK have three sub-forms (coded 1, 2 and 3), each set of sub-forms derived from three genes. For JNK, each sub-form has multiple isoforms; four for JNK1, four for JNK2 and two for JNK3 [20]. Thus far, four sub-types have been cloned for p38; α, β, γ, δ [21].

The mechanics of MAP kinase signalling are covered by several recent reviews with details of triggering stimuli and substrates [20, 22–24]. Their perceived roles in cell physiology are evolving, but in general p38 is viewed as an osmotic response element [25], JNKs respond to several forms of cellular stress and have become the archetypal stress-activated protein kinases (SAP kinase) [20] and ERKs are primarily regarded as growth factor signalling kinases [22]. It subsequently became clear, however, that both the ERK and p38 kinase groups also respond to cellular stressors (for examples, see [26–29]), so all three MAP kinase groups could turn out to be SAP kinases under certain conditions. The p38 osmotic stress response discussed below is typical of one type of MAP kinase stress response, in that the external stimulus causes adjustments of gene transcription which alter the cellular phenotype. As is described below, the p38 kinase signalling pathway is not responsible for all of the cellular responses to osmotic stress and possibly it could be discovered that the other MAP kinases act in concert to produce the full range of cellular adaptation [30]. Other MAP kinase-initiated responses involve a shift in cell cycle to favour proliferation. Such responses have been extensively studied in oncology [31–33].

Sorbitol pathway

In diabetes glucose is the principal sorbitol pathway substrate and it is converted to sorbitol by the enzyme aldose reductase, with oxidation of NADPH, followed by oxidation of the sorbitol to fructose by sorbitol dehydrogenase, with reduction of NAD^+. Under physiological conditions the pathway also uses glucose and it functions to produce uncharged intracellular osmolytes, as follows.

Renal tubular cells at the medullary extremity of the loop of Henlé are bathed in hypertonic interstitial fluid and the tubular cell compensates for this osmot-

ic stress by producing sorbitol from glucose by aldose reductase [34]. Exposure of kidney cells in culture to increments in tonicity of their culture medium stimulates transcription of the mRNA for aldose reductase; with increased enzyme activity and sorbitol accumulation this increased transcription is arrested, attaining a new steady state appropriate to the extracellular osmolality [35]. Other molecules also contribute, in particular *myo*-inositol, betaine and glycerophosphorylcholine and the amino acids taurine and aspartate could also be increased intracellularly on exposure to hypertonic extracellular fluid [36–38]. This phenomenon is not restricted to kidney cells, similar changes in vitro are seen in lens epithelium [39] and the retinal pigment epithelium [37]. It is, therefore, probable that the capacity to increase aldose reductase expression and produce graded amounts of sorbitol in response to hypertonicity is a conserved and a fundamental property of nucleated cells. The identity of the "osmosensor" which initiates this cellular reflex is a mystery, but is considered later.

The involvement of the sorbitol pathway in diabetes is not as simple as the above account might imply. Under certain conditions the pathway could be driven by both osmotic stress and exaggerated concentrations of substrate; raised intracellular glucose resulting from exposure to hyperglycaemia of cells whose glucose uptake is independent of insulin. There is coincidence between insulin-independent glucose uptake and susceptibility to complications [7]. Thus, the situation that pertains in the lens probably exemplifies its most extreme manifestation in diabetes. In diabetes the lens can contain extremely high concentrations of glucose, to which the lenticular epithelium (the site of aldose reductase) is permeable. This is associated with accumulation of sorbitol, which leaks out into the aqueous and vitreous, contributing to an osmotic stress of the lenticular epithelium and increasing expression of aldose reductase [40, 41]. Thus, these combined influences induce aldose reductase and drive a large flux of substrate through its pathway. The extent of osmotically driven aldose reductase expression will vary in other tissues but this will be augmented by raised intracellular glucose, where present. Under these conditions, the cell is forced to produce sorbitol in excess of that required to buffer extracellular tonicity, producing an intracellular osmotic stress. That this occurs is shown by such tissues reducing their content of *myo*-inositol, by reducing expression of its carrier protein [42, 43]. This must compensate for the intracellular stress imposed by the accumulated sorbitol but it is not possible to judge the efficacy of this compensation. The signalling mechanisms for these processes are being explained by analogy with yeast and by studies on renal mechanisms.

Yeast (*Saccharomyces cerevisiae*) responds to osmotic stress by autophosphorylation of at least two cell-surface osmosensors. These then activate a signal cascade upstream of a MAP kinase called HOG1 (high osmolarity glycerol) which, as the name implies, results in reduction of glycerol-3-phosphate to produce glycerol as an intracellular osmolyte [44–46]. This sequence of events is analogous to activation of a mammalian MAP kinase, known as p38, though the mammalian osmosensors have not been characterised. Osmotic challenge of kidney cells induces p38 with follow-on activation of immediate early genes. These almost certainly include some of the transcription-dependent osmoprotective events triggered by hypertonicity [47] but this pathway does not appear to induce aldose reductase [48]. It is likely, therefore, that osmotic stress can activate more than one MAP kinase in susceptible tissues. The extent to which osmotic stress participates in the pathophysiology of diabetes will vary with control of glycaemia. It is, however, equally possible that intracellular osmotic stress, as defined above, is also capable of activating MAP kinases and such an event is likely to occur wherever depletion of *myo*-inositol is associated with sorbitol accumulation in nerve [49, 50], kidney [51, 52] and retina [53–55]. Differential examination of the influences of dehydration and glucose in the kidney shows different pathways signalling the two stimuli [56, 57]; this could imply differential activation of multiple MAP kinases.

Non-enzymatic glycosylation of proteins and receptors for advanced glycosylation end-products (RAGE)

It is clear that there are two generic mechanisms by which non-enzymatic glycation of proteins can cause dysfunction. Firstly, the process changes the protein so that its function within its own domain is altered. Examples are altered physical properties of long-lived extracellular proteins forming connective tissues [58] and basal lamina [59, 60], alteration of intracellular structural proteins [61, 62] and compromise of intercellular communication [63]. These can also be modified by short-term Amadori reactions or by long-term advanced glycation products with cross-linking. The second generic outcome involves non-enzymatic glycation of circulating proteins, which then interact with cell surface receptors, RAGEs. These receptors probably exist to facilitate the uptake and clearance of these glycosylated proteins but when stimulated to excess, they can mediate adverse cellular events. Recently it has been shown that ligand binding to RAGE activates MAP kinases [15, 64, 65]. The responses downstream of MAP kinase activation by RAGE have yet to be identified but earlier considerations suggest a phenotypic shift or proliferation.

Oxidative stress

The notion that oxidative stress participates in the development of diabetic complications dates back over 20 years [66]. Detailed chemistry of interactions between oxidatively derived radicals and tissue components is reviewed elsewhere [67–70]. Plasma lipid peroxides signal oxidative stress [71–73] and this could represent a forerunner of atherosclerosis but it is intracellular oxidative stress that contributes to changes in the cells participating in neuropathy, nephropathy and retinopathy. Furthermore, the oxidation of glucose and the production of reactive intermediates, which interact with intracellular proteins, could well provide a powerful input to the early development of these complications [18, 74].

Pragmatically, there are many studies in diabetic rats showing that treatment with antioxidants attenuates or prevents the development of abnormalities of biochemistry or function that are relevant to complications (for early examples, see [75–80]). In patients, there are fewer studies with traditional antioxidants [78, 81, 82] but it is probable that the beneficial effects of α-lipoic acid [83–85] derive from its antioxidant properties.

There is a large and accumulating literature linking cellular oxidative stress with activation of MAP kinases [27, 86–92]. Although none of this work has been done in diabetes, the markers of oxidative stress are similar in diabetes to those associated with the forms of pro-oxidant treatment used in the studies referred to above.

In general, this work is descriptive and cumulative, in that each new study seems to increase complexity, rather than reveal patterns. Some clarity is, however, evident. Those oxidative stresses that decrease intracellular reduced glutathione (GSH) activate ERK, rather than JNK or p38, at least in fibroblasts [88]. Decreased concentrations of GSH are a consequence of increased flux through the polyol pathway in diabetes [12, 93, 94], probably because aldose reductase competes with glutathione reductase for NADPH. Thus, there is a possible link between the sorbitol pathway, oxidative stress and ERK activation, which could explain the activation of ERK seen in nervous tissue in diabetic rats (Fig. 2). Other apparent patterns conflict, though it is likely that different cell types have different responses. Thus, hydrogen peroxide activated all three MAP kinases (ERK, JNK, p38) in neonatal ventricular myocytes [90] and in several other cell types [27]. It also activated p38 (the other MAP kinases were not studied) in endothelial cells [87], but in mouse fibroblasts JNK was completely refractory to hydrogen peroxide [95]. As the high concentrations of hydrogen peroxide used in these studies are primarily of value for proof of principle, we now need a systematic examination of the effects of realistic oxidative stress to explain its influence on MAP kinases in diabetes.

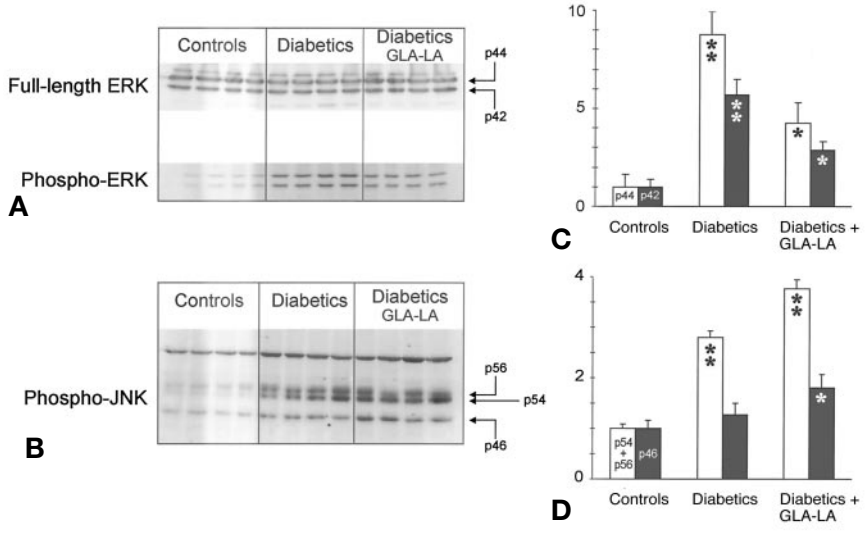

Fig. 2 A–D. Activation (phosphorylation) of ERK and JNK in dorsal root ganglia of diabetic rats, plus effects of treatment with a diester of γ-linolenic and α-lipoic acids (GLA-LA; 200 mg · kg^{-1} · day^{-1} p. o.). Streptozotocin-diabetic rats were either untreated or treated as described above for 8 weeks. Dorsal root ganglia were removed bilaterally at L_4 and L_5, pooled and processed for western blotting exactly as described elsewhere [102]. Each lane contains protein from the four ganglia from a single rat. Blots (**A**, ERK and **B**, JNK) were exposed to antibodies generated against non-phosphorylated epitopes of ERK ("full-length") and JNK (not shown), which revealed no differences between treatments. Exposure to antibodies against phosphorylated epitopes showed considerable activation in untreated diabetic rats (lanes 5 to 8 and bar charts, (**C**, ERK and **D**, JNK) in which phospho-reactivity is normalised to non-phospho-reactivity to correct for any variation in total protein content). Treatment with GLA-LA attenuated ERK activation (**A** and **C**), but enhanced activation of JNK (**B** and **D**)by diabetes. For all methods and sources see [102]; * $p < 0.05$, ** $p < 0.01$ vs untreated controls

An alternative approach, perhaps with even more value, will come from examination of the influence of various antioxidants on MAP kinase activation both in the presence of specific pro-oxidants and in diabetes. This approach also brings another dimension to this analysis. Activation of MAP kinases in cellular stress possibly forms part of the chain of damage inflicted on the cell by this stress or they could form part of the protective response of the cell. Clearly, the examples considered earlier in the context of osmotic stress illustrate MAP kinase responses in the latter category but when activated by oxidative stress, their identity as criminal or policeman is much less clear. One study shows the complexity of this issue. HeLa or Hep G2 cells treated with butylated hydroxyanisole (BHA), which generates intracellular hydrogen peroxide, show brisk activation of ERK2, followed by a slower activation of JNK1. Pretreatment with antioxidants (*N*-acetyl-L-cysteine, glutathione or vitamin E) attenuated the ERK2 response but not JNK1 activation [89]. This suggests that ERK activation forms an early component of the damaging effect of oxidative stress, whereas JNK activation could form part of a protective cellular response. These possibilities receive support from experiments on diabetic rats in the author's laboratory (Fig. 2). Dorsal root ganglia from rats with streptozotocin-induced diabetes show strong steady-sate activation of both ERK and JNK ([96] and Fig. 2). In rats treated throughout their diabetes with a diester of α-lipoic acid and γ-linolenic acid (GLA-LA), which has antioxidant properties, the ERK response was considerably attenuated but the JNK response was greatly enhanced. As GLA-LA is protective against a number of functional and biochemical defects in diabetic rats [97], these observations support the hypothesis that JNK activation is protective, with the exaggerated activation indicating increased antioxidant protection. The role of ERK remains ambiguous, in that reduction of its activation by an antioxidant could imply that phospho-ERK is damaging but it could also imply that it is a sensitive protective element and that its reduced activation shows the attenuated oxidative stress associated with the antioxidant treatment.

Protein kinase C

It was reported 10 years ago that exposure of renal glomeruli [98] or endothelial cells [99] to high glucose activates protein kinase C (PKC). These observations were refined to show activation of multiple sub-types of PKC in retina of diabetic rats, together with raised concentrations of diacylglycerol; these changes were restored to normal by insulin treatment [100]. The mechanism linking raised glucose to PKC activation could involve oxidative stress because treatment with either vitamin E or probucol prevents activation [101] and associated retinal hyperaemia [102]. The important link between these phenomena in retinal vasculature and the proliferative phase of retinopathy came with the finding that the activated PKC is involved in the generation of vascular endothelial growth factor (VEGF) [103].

The next important step in the development of understanding of these processes in the kidney came with the observation that calphostin C, a PKC inhibitor, prevented activation of MAP kinase and phospholipase A_2 in mesangial cells cultured in high glucose [104]. The polyol pathway was also tied into these phenomena with the observation that an aldose reductase inhibitor prevented the activation of phospholipase A_2 in glomeruli from diabetic rats [105]. More directly still, an aldose reductase inhibitor was shown to inhibit the activation of PKC and the increase in transforming growth factor β (TGFβ) production in human mesangial cells cultured in high glucose [106].

The co-activation of PKC and MAP kinases in vascular cells maintained in high glucose [107] reinforces the notion that the two are linked. There is a growing literature outside of diabetes research showing that PKC can activate MAP kinases and that PKC inhibitors can prevent their activation by a range of stimuli (see, for example, [108]) [33, 90]. Thus, the observations cited above suggest that, in vascular tissue, activation of PKC (possibly amplified by increases in its expression) occurs in diabetes by oxidative stress and exaggerated flux through the polyol pathway. Protein kinase C and diacylglycerol provide haemodynamic abnormalities by impairment of prostanoid and nitric oxide production. The production of VEGF is stimulated synergistically by activated PKC and hypoxia derived from the haemodynamic disturbance. Increased TGFβ provokes increased synthesis of extracellular matrix materials. The activation of MAP kinases by PKC – as well as through the other pathways described earlier – is implicated in the transcription-dependent changes and phenotypic switches underlying these and probably other undisclosed phenomena. These considerations support the exploration of the potential benefits of a selective inhibitor of PKC sub-forms as agents targeted at diabetic retinopathy [109].

Diabetic neuropathy

It should be emphasised that the changes referred to above are vascular in origin and could well be central to the abnormalities associated with nephropathy and retinopathy. It is in these tissues where altered production and glycosylation of extracellular matrix have a profound effect and where microvascular pathology is instrumental in the development of dys-

function. In peripheral nervous tissue of diabetic rats there is no evidence of steady-state activation of any of the sub-forms of PKC [110–113]. This indicates that there are fundamental differences in the transduction of glucose effects in nervous tissue from those in retina and kidney. There are those who have argued for vascular causation of neuropathy through endoneurial ischaemia of peripheral nerve trunks [114–116]. It is, however, difficult to reconcile such a simple hypothesis with a condition in which sensory defects can be present without symptomatic problems in motor fibres of the same trunk [117]. Of course, overwhelming evidence implicates vascular dysfunction in the development, or maturation of neuropathy in diabetes, but nerve ischaemia might more accurately be viewed as an accelerator (Fig. 1), than as a primary cause. Earlier metabolic changes in diabetes make nerves more susceptible to ischaemic damage [118]. Thus, we must identify the glucose transducer in peripheral nerve and find phenomena which bias the damaging effect of glucose towards sensory fibres.

Neurones are terminally differentiated, so that the MAP kinases do not subserve transcriptional changes associated with proliferation; instead they communicate signals which adjust neuronal phenotype [119, 120]. Nevertheless, a large amount of work in this area has used PC12 cells. These are a rat pheochromocytoma cell line and do proliferate, so that not all findings extrapolate to adult neurones in vivo, and it is this phenotype that enters the pathway to damage in diabetes. Mitogen-activated protein kinases are involved in the signalling cascade activated by neurotrophic factors [121, 122] and, because neurotrophins have effects on developing nerves which differ considerably from those on adult neurones, there is need for further caution in extrapolation. Some facts, however, are clear.

Mitogen-activated protein kinases are activated downstream of a cascade initiated by phosphorylation of the tyrosine kinase domains of the neurotrophin tyrosine kinase receptors, which proceeds through adaptor proteins and small GTP-binding proteins of the Ras family, phosphorylating Raf and activating the MAP kinase module [108, 123]. Extracellular signal regulated kinase is the end-stage MAP kinase in this cascade and it phosphorylates both nuclear and cytoplasmic substrates.

In neurones JNK is activated by a variety of stimuli, including growth factors, trophic factors, cytokines, ultraviolet light and HIV-1 [124–126]. There is little evidence of activation of p38 in neurones and certainly no indication of stress-induced phosphorylation of this kinase. Activation of JNK and ERK by growth/trophic factors appears to be differential, both with respect to the activating stimuli and the involvement of the Ras GTP binding proteins [124]. Nerve growth factor (NGF) is capable of activating both ERK and JNK under different conditions and with different results. Nerve growth factor interacts with two different receptors in neurones as part of its physiological action; these are the high-affinity tyrosine kinase receptor, trkA, and the low-affinity receptor, $p75^{NTR}$. Where these receptors act in concert, the signalling pathway described above through ERK predominates and the NGF-supported phenotype is maintained, for example in non-myelinated nociceptors [127–129]. A different picture emerges when the p75 neurotrophin receptor is present without trkA, because under these conditions NGF activates JNK and cell death can be a consequence [130, 131]. Introduction of trkA removes the link to cell death [132].

Not surprisingly, these considerations have led to the view that, whereas ERK appears to exert a role in the normal physiological signalling of healthy neurones, JNK has some sinister role. In sensory nerves of diabetic rats both are activated (Fig. 2 and [96]) but the extent to which this happens in human nervous tissue in diabetes is still to be shown. The consequences of persistent activation of MAP kinases in diabetes are also unknown as yet, though some speculations are possible. Firstly, the consequences possibly differentiate between different classes of neurone, because ERK is strikingly absent from the cell bodies of motor neurones [96]. Accordingly an involvement in dysfunction could explain the predisposition of diabetic neuropathy for sensory fibres. Secondly, persistent high-level activation of ERK could make the transcription factors that are its normal substrate, when activated by neurotrophin trk receptors, refractory to neurotrophin stimulation. This is speculative, but some conflict is certainly possible and part of the signalling process for NGF requires its capture, internalisation and retrograde axonal transport to the neuronal cell body, processes which are impaired in diabetes [133, 134]. It is impossible to reconcile the reduced concentrations of NGF available to peripheral neurones in diabetic rats [135] and the impaired receptor function [136, 137] with any assumption that ERK is activated only as part of the NGF signalling cascade. Clearly, in diabetic rats, ERK activation in neurones is driven by something other than neurotrophins; but it is not driven by insulin, which, along with NGF, was the first physiological ERK activator to be identified [138].

We do not know what transcription signals are altered by the persistently activated ERK in diabetes; indeed it could be that persistent activation of a signal that might normally be pulsatile could render downstream processes refractory. There is good evidence that the persistent activation of JNK causes phosphorylation of inappropriate substrates, in particular non-nuclear substrates such as neurofilaments, where it could be the direct cause of hyperphosphorylation [96]. Hyperphosphorylation of neurofilaments is associated with filamentous tangles, axodendritic swell-

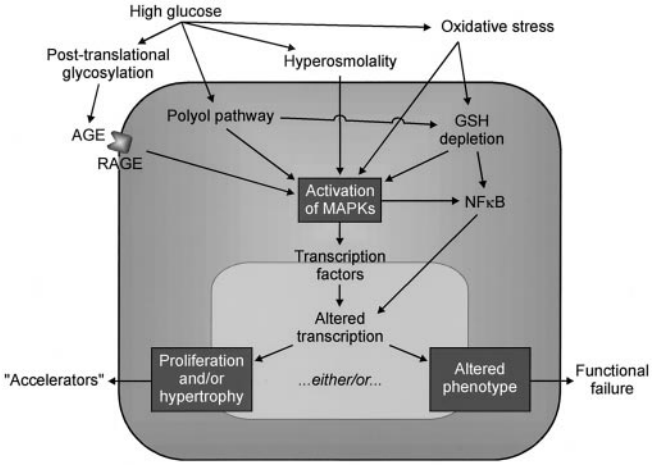

Fig. 3. Synopsis of the hypotheses advanced in this review. Hyperglycaemia exerts several influences on cells, which can synergise by activation of mitogen-activated protein kinases (MAPKs). By adjustment of activation of transcription factors, these can alter the balance of gene expression, leading to a spectrum of changes from cellular proliferation, altered production of extracellular materials or a dysfunctional change in phenotype. NFκB, nuclear factor kappa B

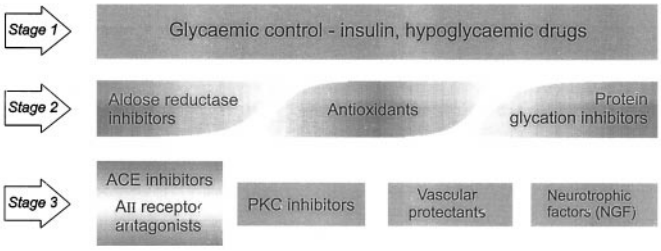

Fig. 4. Schematic illustrating pharmacological defence against glucose and the causation of diabetic complications. This can be considered in three stages, with Stage 1 represented by protection of all tissues against hyperglycaemia; inevitably this will fail at times. Stage 2 indicates protection by removal of a relatively specific biochemical abnormality and the shaded edges of these compartments indicates the overlap referred to in the text. It is implicit that Stage 2 will protect more than one tissue/organ. In Stage 3, targeting is more specific for individual complications or even, as exemplified by nerve growth factor (NGF), a sub-division of the peripheral nervous system. A breach of the protection offered by insulin requires a second line of defence against a range of insults and, so far, no single drug gives such comprehensive cover. A clinical trial of any single agent in Stage 2 (antioxidants, aldose reductase inhibitors, anti-glycation agents) will leave large gaps in the Stage 2 defences, which could prejudice trial outcomes

ing and neurodegeneration associated and is present in a range of diseases, including human diabetic neuropathy [139, 140]. It is, therefore, possible that JNK activation could be pathogenic through neurofilament phosphorylation in several disease states. Obviously, it is difficult to reconcile these speculations with the finding that a drug with many beneficial effects in diabetic rats [97] appears to amplify activation of a MAP kinase that could be damaging. Our understanding of these phenomena is in its infancy. What little we do understand, as covered by this review, is summarised in Figure 3.

Drug targets?

The final consideration is the possibility that these MAP kinases could serve as useful targets for novel drugs targeted at diabetic complications. The first reaction is that they are too ubiquitous in both distribution and function so that modulation would have too many side effects. The notion that an antagonist of PKC could have therapeutic value might, however, have been ridiculed 10 years ago, whereas the development of understanding of different sub-forms of PKC has produced a selective antagonist that is now in clinical trial for retinopathy. Figure 4 illustrates the problems of developing new drugs against complications, a venture that has not been strikingly successful. The extreme refinement of drug action, as exemplified by nerve growth factor, gives an agent whose targets are restricted to a sub-population of affected neurones in diabetic neuropathy. Such an agent might have been useful as part of a battery of drugs, though in isolation it was not possible to show any efficacy and clinical development has ceased. In contrast, angiotensin converting enzyme inhibitors have been successful in slowing the progression of nephropathy because they control a major independent accelerator of the complication. Nevertheless, Figure 4 makes it clear that an ideal agent will have a broad spectrum of action. The potential involvement of MAP kinases in the full range of actions of glucose against all of the tissues responsible for the late complications of diabetes suggests that they should be given serious consideration as drug targets.

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