PERSPECTIVE

Role of glucose regulatory mechanisms in diabetic retinopathy

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The macrovascular and microvascular complications of diabetes have far reaching consequences for sufferers of this disease. In particular, retinal microvascular damage causes diabetic retinopathy which is the single most common cause of blindness in the working population of the Western world.

Several factors have been implicated in the mechanism(s) of diabetic retinopathy.^{1,2} These include non-enzymatic glycation, free radical damage, aldose reductase induction, and myoinositol depletion. However, it is clear from the Diabetes Control and Complication Trial (DCCT)³ that a clear correlation exists between glucose control and susceptibility to the complications of diabetes. Microvascular retinal abnormalities increase in incidence with the duration of the disease and the glucose control regimen of individuals. The 74% reduction of diabetic retinopathy in patients who had undergone intensive glucose control in the DCCT illustrates clearly that glucose regulation is linked to the onset and progression of diabetic retinopathy. Glucose availability affects all aspects of cell function including cell proliferation. A greater understanding of the specific events that are involved and the factors that influence cell growth is central to our understanding of the pathophysiology of diabetic retinopathy, in which impaired cell growth and excessive cell proliferation co-exist.

The entry of glucose into the cells is mediated by a family of proteins called glucose transporters. These proteins achieve their effect by means of a facilitative diffusive process. At present there are at least seven different forms of glucose transporters, which are characterised by their tissue distribution, distinctive kinetic properties, and their responsiveness to various stimuli (Table 1).^{4–6}

Although GLUT 1–4 function as glucose transporters, the site at which the glucose molecule interacts with the glucose transporter is slightly different for each isoform,⁷ thus conferring their hexose specificity and the unique kinetic properties of the respective proteins (Fig 1).

The regulation of the expression and the subcellular distribution of the glucose transporters occurs in response to growth factors, insulin, glucocorticoids, and as changes in the ambient glucose level occur.⁸ In situations of glucose starvation, glucose transport is modified by increasing basal glucose transport⁹ and altering the distribution of glucose transporters.¹⁰ GLUT-4, the insulin responsive

glucose transporter has also been shown to be regulated pretranslationally in response to nutritional status¹¹ and insulin.¹⁰

GLUT-1 protein has previously been detected in endothelial cells and in the retinal pigmented epithelial layer of the rat eye.¹² GLUT-1¹³ and GLUT-3 mRNA,¹⁴ and GLUT-1 protein¹³ have been detected in bovine retinal endothelial cells. We report here on the detection of GLUT-1 and GLUT-3 protein, and GLUT-1 and GLUT-3 mRNA in human retinal endothelial cells (Fig 2). The change in the level of GLUT-3 mRNA has been shown to be regulated by the prevailing glucose concentration in bovine retinal endothelial cells.¹⁴ Mandarino and colleagues¹³ have demonstrated that GLUT-1 mRNA is similarly regulated by high glucose in retinal pericytes. However, the proposal that the absence of any effect on GLUT-1 in endothelial cells may correlate with the relative susceptibility of pericytes is not tenable from these data, as the role of alternative isoforms of glucose transporters – for example, GLUT-3, is not considered. Nutritional regulation of gene expression has been shown to be a specific regulatory mechanism by which insulin secretion from the β cells occurs.¹⁵ This may occur at the level of transcription via a specific regulatory protein, insulin enhancer factor 1 (IEF-1) which binds to the insulin enhancer binding site 1 (IEB-1) or IEB-2,¹⁶ located in the 5' region of the insulin gene.¹⁷ The insulin gene may also be regulated at the level of mRNA stability. Insulin mRNA has been found to have a half life of approximately 30 hours in 3.3 mM glucose, while an increase in the ambient glucose concentration to 17 mM increased the half life of insulin mRNA threefold.¹⁸ Regulation of insulin mRNA at the level of transcription and/or mRNA stability enables the level of insulin to be finely tuned to physiological demands and the subsequent control of the circulating glucose concentration. In the retina, the response of GLUT-3 mRNA to changes in glucose concentrations¹⁴ may be similarly regulated and demonstrates a further example of nutrient control of gene expression. This mechanism of regulation has the potential to play a significant role in the homeostatic mechanisms

Table 1 Tissue specificity of facilitative glucose transporters

Designation	Tissue location
GLUT-1	Human erythrocytes, blood-brain barrier, placenta, retina, transformed cells (in vitro)
GLUT-2	Liver, pancreatic β cells, kidney, intestine
GLUT-3	Brain, retina, placenta, kidney
GLUT-4	White adipose cells, brown adipose cells, red and white muscle, heart muscle
GLUT-5	Small intestine, kidney, skeletal muscle, adipose tissue, retina
GLUT-6	Pseudogene
GLUT-7	Liver/microsomal transporter

Figure 1 Proposed model for the binding of β -D-glucose to GLUT-3 and GLUT-4.⁷

Fig 2A

Fig 2B

Figure 2 Detection of GLUT-1 and GLUT-3 in human retinal endothelial cells by (A) immunohistochemistry (magnification GLUT-1 $\times 550$ (upper) and GLUT-3 $\times 250$ (lower)) and (B) by northern hybridisation. Cytospins of human retinal endothelial cells (HREC) were prepared. GLUT-1 and GLUT-3 protein were identified by specific staining with a polyclonal antibody (East Acres Biologicals, USA) with diaminobenzidine hydrochloride detection. Total RNA was extracted from HREC and resolved on a denaturing agarose gel. The RNA was then transferred to a nylon membrane by capillary blotting and hybridised with a GLUT-1 or GLUT-3 radiolabelled cDNA probe.¹² The cells expressed a 2.8 kb GLUT-1 mRNA and a 4.1 and 3.2 kb mRNA specific for GLUT-3.

implicit to the changing glucose concentrations that are associated with diabetes.

Specific abnormalities of glucose transporters have been identified in diabetes.¹⁹ The distribution of GLUT-1 protein has been shown to be altered in the retina of patients suffering from diabetic retinopathy.²⁰ GLUT-1 was located in the neovascular endothelium of the diabetic retina and the authors propose a distinct role for GLUT-1 in the barrier properties of the tissue. Similarly, in the brain, GLUT-1 has been proposed to be the most significant contributor to glucose transport at the blood-brain barrier²¹ and has been specifically located in the microvascular endothelial cells of the brain. Interestingly, this glucose transporter isoform has also been shown to be down regulated in rats with experimentally induced

diabetes²² and that this process was the result of post-transcriptional inhibition of glucose transporter mRNA translation.

High ambient glucose levels have been shown to alter second messenger generation in astrocytes, and, by implication, cellular events that are dependent upon the generation of second messengers.²³ Increases in circulating glucose levels which may prevail in the circulation, or in the localised environment of the retina, of diabetic patients may therefore influence cellular responses by altering second messenger generation. Therefore, cellular interactions which are dependent upon second messenger action and which are important for the maintenance of normal microvessel function in the retina may be disrupted. The activation of protein kinase C (PKC) by high glucose concentrations has already been shown to be an important pathway by which glucose toxicity may be mediated in retinal cells,²⁴ and is proposed to be the result of de novo synthesis of diacylglycerol (DAG) which then activates PKC.²⁵ Further evidence to this effect is presented in Figure 3. This illustrates the decrease in DNA synthesis that was observed in retinal endothelial cells when incubated in increasing concentrations of glucose, and the reversal of this decline when the cells were co-incubated with a PKC inhibitor (Roche Products Ltd, Ro 31-8220). The implications of these observations on diabetic retinopathy are twofold. Firstly, vascular cell function, which is characteristically aberrant in diabetic retinopathy, is known to be influenced by factors that are characteristically regulated by PKC, including regulation of DNA synthesis (Fig 3), cell proliferation,²⁶ endothelial permeability,²⁷ and the modulation of the basement membrane.²⁸ Secondly, growth factors^{29 30} mediate the action of intracellular messengers by a complex series of events that includes the intracellular signalling pathways of PKC and DAG.^{25 31}

Growth factors elicit a variety of responses depending upon cell type, concentration, and the presence of other growth factors. The distribution of growth factors in the eye has been reviewed.²⁹ These include acidic fibroblast growth factors (aFGF), basic fibroblast growth factor (bFGF), transforming growth factor- β (TGF β), insulin-like growth factor 1 (IGF-1), platelet derived growth factor (PDGF), platelet derived endothelial cell growth factor (PDEC GF), endothelial cell growth factor (ECGF),

Figure 3 Effect of glucose concentration on DNA synthesis on bovine retinal endothelial cells. Co-incubation of the cells with a protein kinase C (PKC) inhibitor (Roche Products Ltd, Ro 31-8220) indicates that the glucose induced inhibition of DNA synthesis is PKC dependent. Retinal endothelial cells were incubated with a range of glucose concentrations for 16 hours. The cells were then pulse labelled with ^3H -thymidine for 6 hours after which time they were harvested onto a nitrocellulose filter and the radioactivity that was incorporated by the cells was subsequently determined using a Matrix₉₆ (Packard).

Fig 4A

Fig 4B

Figure 4 Detection of vascular endothelial cell growth factor (VEGF) in human retinal endothelial cells (A) (magnification $\times 550$) and leucocytes (B) (magnification $\times 250$) using immunohistochemistry. Cytospins were prepared of the respective cell types. VEGF was then visualised by immunohistochemistry using a VEGF specific antibody (PreproTech, New Jersey, USA). Specific binding was visualised using diaminobenzidine hydrochloride.

Figure 5 Detection of vascular endothelial cell growth factor (VEGF) in human retinal endothelial cells (a) and leucocytes (b) using reverse transcriptase-polymerase chain reaction. Total RNA was extracted from retinal endothelial cells and leucocytes. A cDNA was prepared using the enzyme, reverse transcriptase, and an oligo dT. The three forms of VEGF, denoted VEGF₁₂₁, VEGF₁₆₃, and VEGF₁₈₉, correspond to the alternatively spliced forms of the VEGF gene. Using primers specific to the region flanking the spliced area of the gene,³⁵ three products were amplified using the polymerase chain reaction of 403, 535, and 603 base pairs respectively.

vascular endothelial cell growth factor (VEGF), and epidermal growth factor (EGF). The growth factors that are produced within the eye may act in an autocrine and/or a paracrine fashion to achieve their characteristic effects of cell growth, proliferation, and migration. These cellular responses are all known to be associated with diabetic retinopathy in general and specifically in neovascular eye disease.³⁰ The high concentrations of glucose that are

reached in diabetes mellitus can stimulate the transcription of genes coding for growth factors – for example, bFGF in vascular smooth muscle (VSM) cells.³¹ The neovascular associated increase in VSM proliferation poses an interesting link between glucose concentration and the potential growth of new vessels in the eye.

Growth factors may also influence the progression of diabetic complications by altering the innate glucose regulatory mechanisms of the retina. Retinal vascular cells have both insulin and IGF-1 receptors,^{32,33} and thus have the potential for glucose regulation via these growth factors present in the circulation, or in an autocrine/paracrine manner by the locally produced IGF-1.³³ Glucose transport has also been shown to be increased in endothelial cells when exposed to VEGF independent of any insulin effects.³⁴ This is extremely interesting in the light of the expression of VEGF in both retinal endothelial cells and leucocytes (Figs 4 and 5) and also as shown by other workers.^{35–37}

The effect of growth factors on glucose regulation is also evident with PDGF, which increases GLUT-1 mRNA,³⁸ and the level of membrane associated glucose transporters.³⁹ The latter is achieved by an increase in the expression of the oncogenes, c-myc and c-fos, illustrating the link between growth factors, glucose regulation, and cellular proliferation.

The vasculature and the flow of blood through the vessels are also important considerations in the overall mechanism(s) of the disease process as the products released by leucocytes continually bathe the offending cells. Monocytes have been shown to be associated with microaneurysms in the retinas of rats with alloxan induced diabetes,⁴⁰ thus establishing a link between a potential source of growth factors – that is, the monocytes, and the site from which new vessel growth occurs. Mononuclear cells may be dysfunctional at the level of their glucose regulatory mechanisms which is illustrated by the reduced insulin stimulated glucose uptake in mononuclear cells in patients with insulin dependent diabetes.⁴¹ This reduction in the overall level of insulin stimulated glucose uptake may reflect abnormalities of circulating cells which potentially may cause defects in the specific environment of the retina. Additional evidence for the involvement of mononuclear cells in diabetes has come from the cat model of diabetes. Stimulated polymorphonuclear leucocytes generated more superoxide radical at higher rates than their normal counterparts,⁴² which may contribute to the vascular damage and the resulting ischaemia, which has

Figure 6 Factors affecting retinal endothelial cells that may influence the onset and progressive pathology of diabetic retinopathy.

already been demonstrated to exacerbate myocardial ischaemia.⁴³

Thus, circulatory and localised effects must be considered in an overall discussion of the mechanisms of the complications of diabetes and, in particular, of the potential significance of the multifactorial nature of the ensuing severity of diabetic retinopathy (Fig 6). The correlations that have been demonstrated in clinical trial,³ and the manifestation of diabetic complications should focus our thoughts upon the regulation of glucose uptake and metabolism and the characteristic features of the microvasculature which render them so susceptible to glucose homeostasis.

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