Glucose-dependent decreased DNA synthesis in bovine retinal endothelial cells is mediated by protein kinase C iota.

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Diabetic retinopathy (DR) is the most significant cause of visual loss in the working population of the Western world. DR affects the retinal vasculature of people with diabetes. The early stage of DR is characterised by increased vascular permeability, increased blood flow, thickening of the basement membrane and microaneurysm formation. Clinical studies have demonstrated that the concentration of glucose that the endothelium is exposed to is probably the most important determinant of DR severity [2]. The disease is believed to be a consequence of glucose-mediated damage to the microvascular endothelium of the retina, although the precise mechanism by which this occurs remains elusive.

Vascular permeability and changes in endothelial cell growth and proliferation are some of the fundamental changes that occur in retinal microvascular endothelial cells [1]. Protein kinase C (PKC) has been shown to be a potential mediator of these cellular changes [3], and of interest in the investigation of glucose-mediated changes given the known increase in PKC levels in cells exposed to high concentrations of glucose both in vivo [4] and in vitro [5]. We have demonstrated that increased glucose concentrations result in a decrease in DNA synthesis in bovine retinal endothelial cell (BREC) [6,7]. This was shown to occur via a protein kinase C (PKC)-dependent pathway and that it is dependent upon the entry of glucose into the cells [7].

PKC is a family containing at least 11 distinct isoforms [8] that are unique, not only with respect to primary structure, but also on the basis of expression patterns, subcellular localization, activation in vitro and responsiveness to extracellular signals [9]. PKC stimulation triggers a complex cascade of phosphorylation and de-phosphorylation reactions that result in modified cell responsiveness. Therefore we wished to identify the specific isoforms that are involved in the regulation of cell growth and DNA synthesis in BREC.

Bovine REC were cultured in Glasgow’s minimal essential medium (GMEM) as previously described [10,11]. After they had reached approximately 80% confluence they were reseeded into a microtitre plate. We examined how glucose concentration affects BREC growth and DNA synthesis using the Alamar blue assay (Serotech) and ³H-thymidine incorporation. The GMEM was supplemented with either 5 or 20mM D-glucose and with Alamar blue as recommended by the manufacturer. A significant reduction in cell growth occurred after 96 hours and this was also reflected by a decrease in DNA synthesis (Figure 1).

The experiment was then repeated with the addition of a protein kinase C iota antisense oligonucleotide that will specifically inhibit the translation of PKC iota mRNA. BREC that were grown in a normal physiological concentration of glucose (5mM) did not significantly change their growth pattern or their ability to synthesise DNA. However, BREC cultured in high concentrations of glucose, showed the expected profound reduction in DNA synthesis which was abrogated by the presence of the PKC iota antisense (Figure 2).

These data demonstrate a specific involvement of PKC iota in the glucose-dependent decreases in cell growth and DNA synthesis in BREC. The effectiveness of the antisense oligonucleotide demonstrates that there is a requirement for the translation of PKC iota mRNA to mediate this response. PKC iota is a member of the atypical class of PKC isoforms (aPKC) and as such is unique in that the activity of the enzyme is independent of DAG and phorbol esters. This is inconsistent with the glucose-mediated activation of PKC iota that is evident in this study as the glucose-dependent increases in PKC that are mediated via an increased flux through the glycolytic pathway is thought to occur via an increase in the de novo synthesis of DAG [3]. Therefore, an alternate mechanism of PKC iota activation is likely to be involved in the regulation of DNA synthesis and BREC growth that occurs in response to the concentration of glucose. A more detailed study of the mechanism of glucose-induced PKC iota activity is now in progress.

References