TARGETING PKC AND NFκB BY POLYUNSATURATED FATTY ACID MIMETICS IN DIABETIC RETINOPATHY

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SUMMARY

Hyperglycaemia-induced vascular complications of diabetes mellitus continues to cause significant morbidity despite recent advances in therapy. Protein kinase C (PKC) and nuclear factor κB (NFκB) are two key signaling molecules which contribute to the development of diabetic complications, nephropathy, retinopathy and cardiovascular disease. While the omega-3 fatty acids have some protective value in diabetes their use has remained limited. To improve this type of application, our department has undertaken studies to identify the relationship between specific structural elements of polyunsaturated fatty acids and their biological activities. The findings led to the synthesis of a group of novel polyunsaturated fatty acids (PUFAs), one of which (β-oxa 21:3n-3/MP5) inhibits PKCβ activation and another (β-oxa 23:4n-6/MP3) which inhibits NFκB activation. It was therefore of interest to determine the relevance of this technology to the treatment of retinopathy.

This research characterizes the effect of hyperglycaemic conditions on PKC and NFκB activation in bovine retinal endothelial cells (BREC) in culture, and assesses the ability of the novel PUFAs to inhibit PKC and NFκB activation.

The research first established conditions for optimal isolation procedures for preparation of highly pure BREC and culture conditions under which the cells retained the BREC characteristics. The cell preparations were Von Willebrand Factor positive and devoid of pericyte contamination.

A major finding was that BREC expressed PKC α, βI, δ and ε but not βII. This contrasts with previous findings which have reported the activation of the βII isozyme in BREC. Since we were unable to see the expression of PKC βII, the results suggest that previous work had been conducted with BREC contaminated with pericytes.

The data demonstrated that BREC exposed to hyperglycaemic conditions (25mM glucose) showed preferential activation of PKC βI and δ. Hyperglycaemia-induced PKC activation is therefore not generalized to all isozymes, with PKC βI being the most significant.

The results showed that while high glucose alone failed to activate NFκB (measured as degradation of IκBα), it caused a more persistent activation of NFκB in response to tumour necrosis factor α (TNF) suggesting that the pathogenic effects of TNF are amplified by hyperglycaemic conditions. The results are consistent with the recent evidence that TNF is a cytokine involved in the pathogenesis of diabetes.

The findings from the research showed that β-oxa 21:3n-3 preferentially inhibited the activation of PKCβI in BREC cultured under hyperglycaemic conditions. Treatment of BREC with β-oxa 23:4n-6 significantly inhibited the activation of NFκB induced by TNF under hyperglycaemic conditions.

This research not only contributes to a better understanding of diabetic retinopathy but also demonstrates novel ways of targeting these signaling molecules (PKC and NFκB) with the PUFA mimetics, MP3 and MP5.

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