Cannabinoid receptor 2 expression in proximal tubule cells exposed to elevated glucose and albumin

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Introduction. Hyperglycaemia plays a significant role in the aetiology of diabetic nephropathy. Compounded with this, renal tubule cells are also exposed to high levels of albumin in the filtrate, which is caused by damage to the glomerulus. We have previously identified that the cannabinoid receptor 2 (CB2) is present in proximal tubule cells (Jenkin *et al.*, 2010). In renal tubules, hypertrophy is an early indicator of diabetic nephropathy, and previously we have shown that activation of CB2 reduces tubular hypertrophy (Jenkin *et al.*, 2010). Despite this, to date, there has been little investigation into changes in the expression of the CB2 in the proximal tubule under diabetic conditions. The aim of this research was to quantify the expression of endocannabinoid receptor CB2 in proximal tubule cells exposed to high levels of glucose and albumin.

Method. Human kidney (HK2) cells were treated with one of four protocols; a control medium containing physiological normal levels of glucose (5 mM) and no albumin; a high glucose (25 mM) medium with no albumin; a high albumin (1 mg/ml) medium with normal glucose levels (5 mM); or a combination medium composed of high glucose and high albumin. HK2 cells were incubated for 4, 6, 18 or 24 h. Following treatment, mRNA was extracted and DNAse treated. The mRNA was reverse transcribed and the level of the endocannabinoid receptor CB2 mRNA was assessed by real time PCR. Protein expression for the CB2 receptor was investigated using Western blot analysis. Band density was quantified using Image Lab software, and treatment groups were normalized to control treatment. Values were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA.

Results. High levels of glucose did not significantly alter CB2 mRNA expression in HK2 cells. High albumin treatment alone and in combination with high glucose did result in a significant reduction in CB2 receptor mRNA expression at 6 and 18 h compared to control treatment ($P<0.05$, n = 9). CB2 protein expression was reduced compared to control treatment at 6 and 24 h treatment periods again in high albumin treatment alone and in combination with high glucose ($P<0.05$, n = 4).

Conclusion. We have demonstrated that exposure to elevated levels of albumin alone and in combination with glucose significantly reduces mRNA and protein levels of the CB2 receptor in a proximal tubule cell line. High glucose alone did not affect CB2 expression in the proximal tubule. Given that the reduction in the CB2 receptor expression occurred only in treatments containing elevated albumin, this indicates that CB2 may play a protective role in the advanced stages of diabetic nephropathy when the proximal tubule is exposed to elevated albumin levels in the filtrate following glomerular damage due to hyperglycaemia. Further investigation may indicate that this receptor could provide a novel target for the treatment and prevention of diabetic nephropathy.

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