

## Elevated CB1 and GPR55 cannabinoid receptor expression in proximal tubule cells and whole kidney exposed to diabetic conditions

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**Background:** Hyperglycemia has been implicated in the etiology of diabetic nephropathy, which typically leads to elevated albumin levels in the urine as a result of glomerular and tubular dysfunction. A number of molecular targets involved in the pathophysiology of this disease have been identified. Specifically, diabetes mellitus induces an upregulation in the cannabinoid receptor 1 (CB1) and putative cannabinoid receptor g-protein coupled receptor 55 (GPR55) in a tissue specific manner. To date, there has been little investigation into changes in the expression of the CB1 and GPR55 receptors in the kidney and specifically the proximal tubule under diabetic conditions.

**Method:** Human kidney (HK2) proximal tubule cells were incubated with one of four treatments; a control media containing physiological normal levels of glucose (5 mM) and no albumin, a high glucose (25 mM) media with no albumin, a high albumin (1 mg/ml) media with normal glucose levels (5 mM), or a combination media composed of high glucose and high albumin. HK2 cells were incubated for 4, 6, 18 or 24 hours. Following treatment, protein lysate and mRNA was extracted and mRNA was DNase treated. The mRNA was reverse transcribed and the level of CB1 and GPR55 expression was assessed by 'real-time' PCR. Protein expression was assessed by western blot analysis. Further, we characterized whole kidney protein expression of these receptors in Sprague Dawley rats with streptozotocin (STZ) induced diabetes. Protein expression for the CB1 and GPR55 receptors were investigated using Western blot analysis.

**Results:** In whole kidney, CB1 protein expression was significantly increased in diabetic compared to non-diabetic animals. *In vitro* proximal tubule cells exposed to elevated high albumin alone significantly increased CB1 protein expression at 4 hours. Albumin treatment in conjunction with high glucose also lead to significantly elevated CB1 mRNA (6 hour) and protein (4 hours) expression. No alterations to GPR55 expression was found in whole kidney. Specifically in proximal tubule cells high glucose appears to be driving an increase in GPR55 expression. GPR55 mRNA was elevated at 6 and 24 hour treatments with high glucose and high glucose combined with high albumin media. However, only high glucose alone induced significant increases in GPR55 protein expression (6 hour).

**Conclusions:** We have demonstrated that *in vivo* the CB1 receptor is upregulated in whole kidney of diabetic animals. Further, an *in vitro* model of diabetic nephropathy leads to increases to both CB1 and GPR55 receptor expression specifically within the proximal tubule cells. Here we have demonstrated that both CB1 and GPR55 are significantly increased in the renal system under diabetic conditions. Additional investigation may indicate that these receptors may provide useful physiological targets for the treatment and prevention of diabetic nephropathy.