Abstract

Normal abundant dietary sugars such as fructose and glucose can contribute to hypertension and other health issues. To avoid these health complications, many individuals use artificial sweeteners. An equivalent intake of some artificial sweeteners also can lead to hypertension. However, Stevia, a sweetener that is isolated from a Paraguayan plant, was shown in relevant literature to decrease blood pressure. A general increase in the relative expression of both AT1 and PRR in the glucose diet was observed. However, this increase and all other differences in the test groups were not significantly different from the control group. RNA samples were obtained. qPCR designs were developed to measure the relative amounts of PRR receptor and AT1 expression in the test groups.

Introduction

The renin-angiotensin-aldosterone system is located primarily in the kidneys. It is important in regulating blood pressure and blood volume. Renin and angiotensin are the major proteins in this system; they bind to prorenin receptor (PRR) and angiotensin II type 1 receptor (AT1). Binding to these receptors lead to local vasconstriction and hypertension. Hypertension is a serious health problem in the United States, more than 30% of adults have it. A1 expression is increased during hypertension.1 In rats, and increase in fructose & sucrose intake correlated with an increase in AT1 expression and hypertension.2,3,4,5 In both rats and humans, Stevia was shown to decrease blood pressure.6,7 Less information is known about the effects of sugar sweeteners on prorenin receptor expression.

Objectives

To purify mRNA from the rat kidneys and assess its quality. To design and test the efficiency of qPCR primers and probes to measure the expression of prorenin receptor (PRR), angiotensin II type 1 receptor (AT1), and GAPDH (endogenous control). To measure the expression of AT1 and PRR relative to GAPDH in the control and experimental rat kidneys.

Experimental Design

Four groups, four male WKY rats per group
Fed the following liquid diets for 6 weeks: Standard - liquid osmotite diet Glucose - liquid osmotite & glucose Saccharin - liquid osmotite & saccharin Stevia - liquid osmotite & stevia Mixed Diet - liquid osmotite (3 weeks), glucose (1 week), stevia (2 weeks) (2 kidneys)
Measured the relative expression of AT1 and PRR

Methodology

Rat kidneys
Rat kidneys collected and quickly frozen in liquid nitrogen
RNA isolation
Kidney tissue homogenized (Cleavershredder) and RNA isolated (miRvana kit, Ambion)
Reverse Transcription
DNA quantification and then Reverse Transcription (High capacity cDNA kit, Life Tech.)

qPCR & Analysis

qPCR (Gene expression Master Mix, Life Tech.) of cDNA samples and Relative Quantitation using the 2^-ΔΔCT Method

Conclusions

Efficient qPCR systems were established for AT1, PRR, and GAPDH.
RNA was isolated with consistent purity despite a variance in concentration.
No significant differences were found among sample groups, though a slight increase in the relative quantity of glucose was seen for both targets.
The lack of significant differences between diet groups was probably a result of high variance among biological replicates and small sample sizes.

References


Acknowledgements

Undergraduate Research Center Grant
Honors Program Grant
Dr. Theresa Salerno- Mentor
Dr. James Rife- Tissue Collection
Natalie Young – Research partner
Dr. Mary Hadley & their research students

This document is available in alternative formats to individuals with disabilities by calling Accessibility Resources at 507-389-2825 (V), 800-627-3529 or 711 (MRS/TTY).