Effect Of Sweeteners On The Renin-Angiotensin System In Rats

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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 in both rat specimens and humans. The general purpose of this research project was to study the effect of Stevia and glucose on the expression of two key components of the renin-angiotensin-aldosterone system (RAAS): prorenin receptor (PRR) and angiotensin receptor type 1 (AT1). Increased expression of renin and angiotensin can lead to vasoconstriction and systemic hypertension. Their effects are mediated by their binding to PRR and AT1. Therefore, decreases in the expression of these receptor proteins can result in lowered blood pressure. Rats were fed diets supplemented with glucose, saccharin, or Stevia over a six-week period and the kidneys were obtained. qPCR designs were developed to measure the relative amounts of PRR receptor and AT1 receptor. The methods had efficiencies greater than 97% and gave reproducible results. Then the developed methods were used to measure the expression of AT1 and PRR in the different rat kidney samples. 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 than the control group. These results suggest no differences in AT1 or PRR expression for different sweetener diets.

Introduction

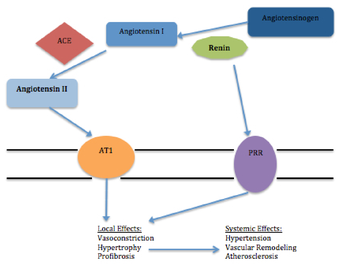


Figure 1. Simplified Diagram of Renin-Angiotensin System

- 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 vasoconstriction and hypertension.
- Hypertension is a serious health problem in the United States, more than 30% of adults have it.[2]
- AT1 expression is increased during hypertension.[1]
- In rats, and increase in fructose & sucrose intake correlated with an increase in AT1 expression and hypertension.[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 sweeteners on prorenin receptor

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

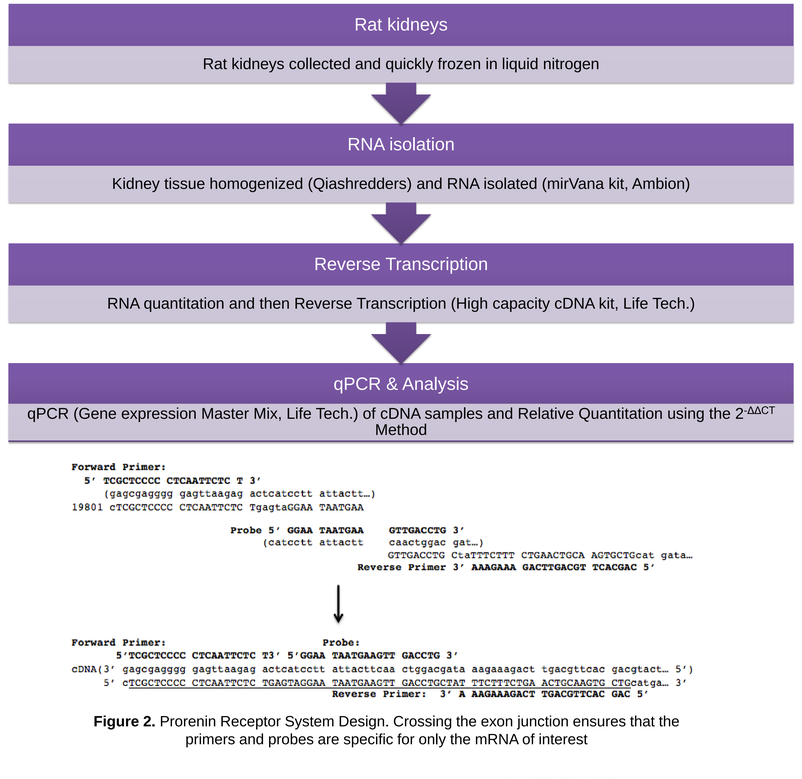
Experimental Design

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

Table 1. Primers and Probes Designed for AT1, PRR, and GAPDH

	Angiotensin II Receptor type 1 (AT1)	Prorenin Receptor (PRR)	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)
Forward	5' GCTGGCATTTTGTCTGGATAAAT 3'	5' TCGCTCCCCCTCAATTCTCT 3'	5' ACGGGAAACCCATCACCAT 3'
Reverse	5' GGGTTGAGTTGGTCTCAGACACT 3'	5' CAGCACTTGCAGTTCAGAAAGAAA 3'	5' CCAGCATCACCCCATTTGA 3'
Probe	5' AGTGATCACCAGGTCAAGT 3'	5' GGAATAATGAAGTTGACCTGC 3'	5' TTCCAGGAGCGAGATC 3'

Methodology

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Forward Primer:
  5' TCGCTCCCC CTCAATTCTC T 3'
     (gagcgagggg gagttaagag actcatcctt attactt…)
19801 cTCGCTCCCC CTCAATTCTC TgagtaGGAA TAATGAA

                          Probe 5' GGAA TAATGAA    GTTGACCTG 3'
                               (catcctt attactt    caactggac gat…)
                                                   GTTGACCTG CtaTTTCTTT CTGAACTGCA AGTGCTGcat gata…
                                                Reverse Primer 3' AAAGAAA GACTTGACGT TCACGAC 5'

Forward Primer:                          Probe:
     5'TCGCTCCCC CTCAATTCTC T3' 5'GGAA TAATGAAGTT GACCTG 3'
cDNA(3' gagcgagggg gagttaagag actcatcctt attacttcaa ctggacgata aagaaagact tgacgttcac gacgtact… 5')
     5' cTCGCTCCCC CTCAATTCTC TGAGTAGGAA TAATGAAGTT GACCTGCTAT TTCTTTCTGA ACTGCAAGTG CTGcatga… 3'
                                         Reverse Primer:  3' A AAGAAAGACT TGACGTTCAC GAC 5'
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Figure 2. Prorenin Receptor System Design. Crossing the exon junction ensures that the primers and probes are specific for only the mRNA of interest

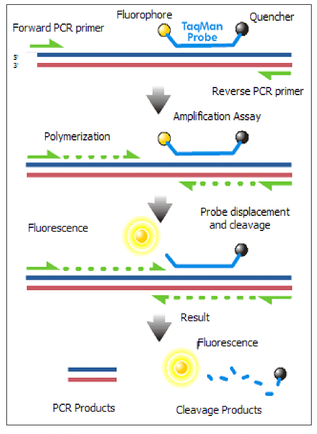

Figure 3. Diagram of qPCR Theory. As polymerization occurs, the Fluorophore is cleaved from the probe and emits measurable fluorescence

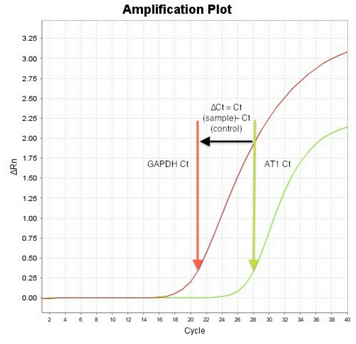

Figure 4. Example Amplification Plot comparing the threshold cycles (C_T) of control and experimental targets. The C_T is the cycle where the fluorescence reaches above baseline fluorescence, generally during the exponential phase of the curve.

→ $\Delta C_T = C_T$ (target) – C_T (control →GAPDH)
→ $\Delta\Delta C_T = \Delta C_T - \Delta C_T$ (Ref → E2)
→ RQ = 2^(-ΔΔC_T)

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.

Results

RNA Purification

Table 2. RNA sample concentrations & purity

Sample	Conc (ng/uL)	A_{260}/A_{280}
E1	592.7	2.01
E2	111.9	1.97
E3	596.7	2.04
E4	439.7	2.02

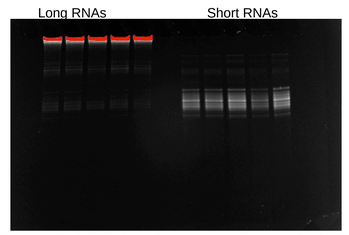

Figure 5. 15% denaturing gel demonstrating the separation of short and long RNAs in the mirVana procedure

Efficiencies of RT-qPCR Methods

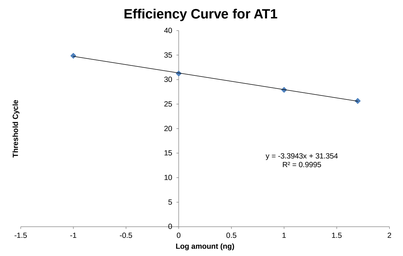

Figure 6. Standard curve to determine AT1 system efficiency. System was 97% efficient.

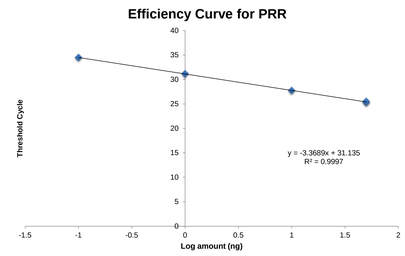

Figure 7. Standard curve to determine PRR system efficiency. System was 98% efficient.

Relative Expressions of AT1 and PRR mRNAs

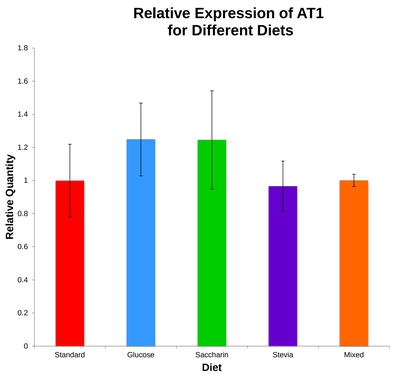

Figure 7. Relative AT1 mRNA quantity for each diet group

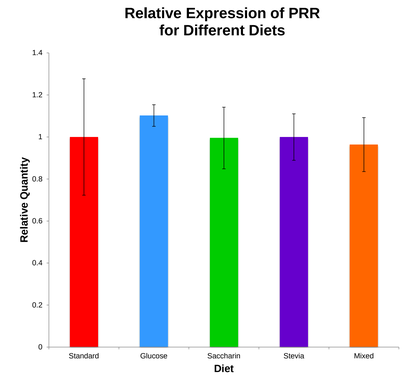

Figure 8. Relative PRR mRNA quantity for each diet group

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