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Changes In Calcium Pathway Gene Product Level Caused by Rosiglitazone Treatment in Neonatal and Adult Rat Isolated Cardiocytes

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Changes In Calcium Pathway Gene Product Level Caused by Rosiglitazone Treatment in Neonatal and Adult Rat Isolated Cardiocytes.

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Abstract: Cardiovascular failure is the number one risk factor in Diabetes and the leading cause of death among diabetics. Earlier studies have shown that, rosiglitazone (Avandia©, GlaxoSmithKline), a widely prescribed anti-diabetic drug, can improve heart performance. To further investigate the underlying cellular basis for heart failure, we have analyzed the metabolic pathways involved in calcium signaling in the heart cell, and their links to gene expression. Studies of rosiglitazone effects on calcium regulation in rat ventricular myocytes during excitation-contraction calcium transient decay rates and SERCA2 gene expression level showed that this drug makes contraction of the cardiomyocytes faster and stronger We have used RT-PCR and western blot to validate results from the microarray analysis for the SR SERCA pump (Atp2a3), ryanodine (Ryr2) and NCX (Slc8a) genes to demonstrate up-regulation of these genes as the source of enhanced contractility accompanying rosiglitazone treatment.

Keywords: Calcium Signaling, Ventricular Cardiomyocytes, Rosiglitazone.

1. Background and significance

More than 65% of deaths in diabetic patients accounted for due to cardiovascular failure. Current studies showed that, especially type II diabetic patients have a higher risk of congestive heart failure. Also studies showed that a widely used prescription drug, rosiglitazone, appeared to improve cardiomyocytes contractility. According to American Heart Association, seven million Americans suffer from Coronary Heart Diseases (CHD) currently; each year more than 1,000,000 Americans die of HD, stroke & related complications.

Preliminary studies on one of the peroxisome proliferators activated receptor-y ligand glitazone, class rosiglitazone, showed an enhancement of cardio-protective performances (improved left ventricular function, anti-inflammatory effects [4].

Earlier studies from our laboratory demonstrated a positive inotropic effect following acute treatment with the drug via the short-term calcium signaling pathway [4]. Recent reports suggest link between long-term use of rosiglitazone and incidence of heart failure and myocardial infarct. [1, 3]. Other ongoing clinical data studies indicate that the heart failure link due to prolonged use of rosiglitazone is not statistically significant [1].

2. Research Design and Methods

The core value of this research design basis on validation of effects of rosiglitazone on gene expression patterns by studying gene product levels using western blot techniques and compare with previous findings.

2.1 Cell Preparation

Neonatal rat ventricular myocytes were isolated and planted on fibronectin coated cover slips and allowed to recover overnight. Rosiglitazone with DMSO carrier treatments were done at 48 hour, 24 hour, 4 hour 2 hour and 1 hour time points. Then cells were lysed using RIPA or QIAGEN buffer for 5 minutes to harvest protein or RNA by scraping.

2.2 RNA and Protein extractions

A protein extraction kit (Bio-Rad) was used to collect the protein and QIAGEN mini assay kit to collect RNA then, the concentration was measured using an RC DC protein assay kit (Bio-Rad).

2.3 Western blotting

SDS-PAGE gel wells were loaded with 15-20 µg of soluble protein samples and run at 125 volt for 60-90 minute followed by a transfer to nitrocellulose membrane at 90 volt for 90

minutes in cold room (4° C). Then the transfer membrane was blocked in a buffer for one hour at room temperature on a shaker and treated with appropriate dilution of primary antibody and incubated overnight in the cold room. On the next day, the blotted membrane was washed 3 times for 10 minutes using TTBS and secondary antibody-HRP was added for 60 minutes in room temperature rocking. Then the blot was washed again 3 times for 10 minutes and a detection reagent (ECL) was added and developed on film in the dark room to get the protein binding on the blot.

The processed film was scanned and analyzed using an ImageJ (NIMH) software gel analysis toolkit to plot the normalized expression levels of gene products.

3. Results

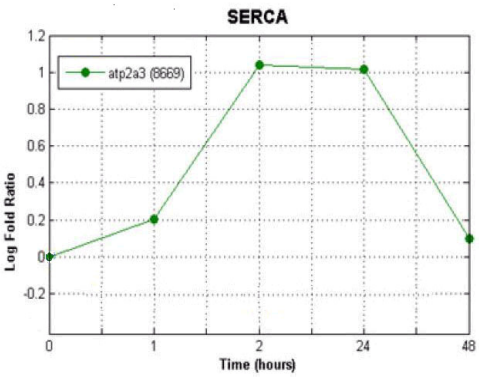

Figure 1. GE/Amersham array result for SERCA expression profile

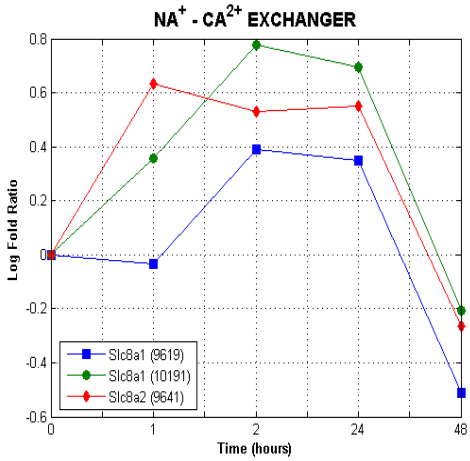

Figure 2. GE/Amersham array result for NCX1 isoforms profile

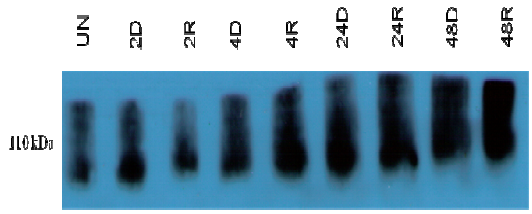

Figure 3. Western blot for SERCA ATPase pump

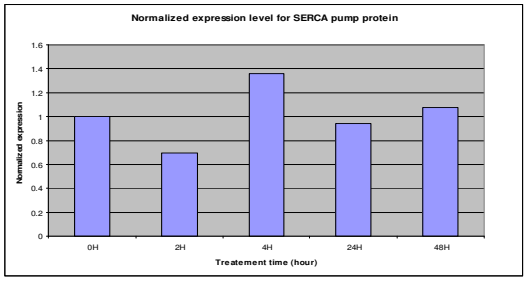

Figure 4. ImageJ profile for SERCA ATPase pump

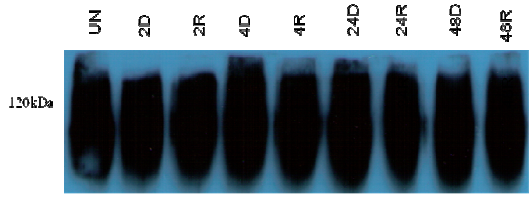

Figure 5. Western blot for NCX1 channel

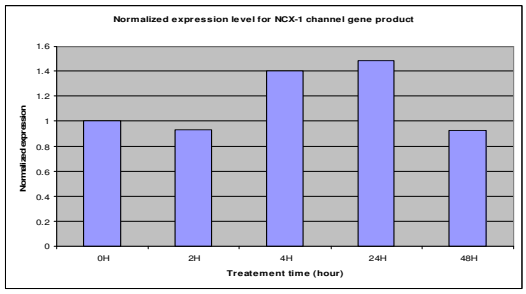

Figure 6. ImageJ profile for NCX1 channel

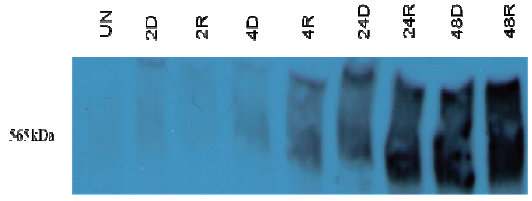

Figure 7. Western blot for Ryr-2 receptor

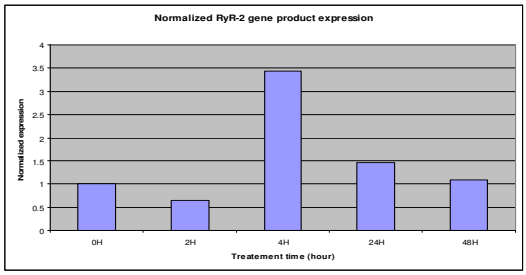

Figure 8. ImageJ profile for Ryr-2 receptor

Figure 9. Western blot for GAPDH as a loading control

4. Discussions and Conclusion

Current result findings (figure 4) for SERCA expression change due to rosiglitazone treatment on ventricular cardiocytes correlates with prior lab result of microarray (figure 1). Similarly western blot results for NCX and Ryr (figure 6 and 8) shows agreement with previous lab results (figure 2).

SERCA2 ATPase pump protein level exhibits a significant increase at the 4 hour rosiglitazone treatment (in general agreement with microarray experiments).

NCX1 channel protein level does not show a clear consistency in regulation; however there was an increased activity at the 4hour treatment point (in general agreement with microarray data).

RyR2 receptor protein level showed a significant expression increase at the 4 hour rosiglitazone treatment point (in general agreement with microarray data).

Enhanced contractility following rosiglitazone treatment appears due to increased calcium transient amplitude (from Ryr2 gene expression increase) and faster return to resting calcium levels (from SERCA2 and NCX gene expression increases) [5].

5. Future Direction

Determine whether SERCA, NCX and Ryr2 protein expression levels correlate with RNA measurements from qPCR.

Compare functional data (contractility assay, calcium transient analysis) with Western **blot data from the same cell population**

6. References

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