What is the myocardial implication in RAAS-regulated hypertension? A closer look at the NKA, NCX and Ca\(^{2+}\) reuptake machinery in the left ventricular myocytes.

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### Abstract

Introduction: Renin Angiotensin-Aldosterone System (RAAS), a hormonal system that regulates fluid retention, sodium-potassium, volume homeostasis and blood pressure. Objective: The present study characterizes circulation of RAAS in (mRen2)27 transgenic model of hypertension, to understand the machinery for cardiac excitation-contraction coupling and arrhythmias in RAAS-regulated high blood pressure. Methods: Analysis of cardiomyocytes showed imbalance in the expression of RAAS receptors (AT\(_R\), AT\(_R\) and MAS), Na\(^+\)/K\(^+\) ATPase (NKA) pump, Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) system and alteration of intracellular Ca\(^{2+}\). Results: Protein expression for AT\(_R\), AT\(_R\) and Ang1.7-mediated-MAS receptors were significantly reduced in the cardiomyocytes of (mRen2)27. The relevant role of NKA and NCX in Na\(^+\) homeostasis was hypothesized that an increase in NKA isoforms (c1 & c2) would suggest a surge in the NCX exchanger to maintain Na\(^+\) influx in cardiomyocytes. The protein expression of the NKA isoforms in the left ventricular myocytes suggests a two-fold increase in (mRen2)27. Further, RT-PCR showed that there is a decrease in mRNA profile for sarco/endo-plasmatic reticulum Ca\(^{2+}\)-ATPase (SERCA) Atp2c-2, suggesting a decrease in the slow twitch of Ca\(^{2+}\) reuptake in sarcoplasmic reticulum which results in an increased intracellular Ca\(^{2+}\) and cardiac excitation-contraction coupling in the hypertensive rodents. Conclusion: The data suggests that the optimum role of NKA, NCX and SERCA, in handling of Na\(^+\) and Ca\(^{2+}\) in ventricular myocytes, is different in RAAS-induced hypertension.

### Results

![Image of results](image)

**Figure 2:** Protein expression of RAAS receptors A) AT\(_R\), B) AT\(_R\) and C) MAS profile in the Heart Left Ventricles (LV) normalized to β-actin protein expression and expressed as intensity ratios compared to SD. Protein expression results suggest a three-fold increase in mRen2-AT\(_R\). There was a three-fold decrease in AT\(_R\) and twofold decrease of MAS in the hRen2 transgene.

**Figure 3:** Protein expression of Na\(^+\)K\(^+\) transporter subunits A) alpha 1 and B) alpha 2 profile in the Heart Left Ventricles (LV) normalized to β-actin protein expression and expressed as intensity ratios compared to SD. Protein expression results suggest a twofold increase in mRen2-AT\(_R\) alpha 1. There was a three-fold increase of Na\(^+\)K\(^+\) alpha 2 in the hRen2 transgene.

**Figure 4:** RT-PCR expression of mRNAs. Relative gene expression of A) MAS and B) Slc8a1 profile. mRNA for MAS is significantly lower in LV of animal compared to control (*p-value 0.0222) whereas one-fold increase of Slc8a1. (mRen2)27 transgenic hypertensive

### Methods

**Blood pressure** was recorded using NIBP to determine baseline blood pressures for normotensive and hypertensive animals.

**Western Blot** was used to visualize and quantify protein expression of RAAS receptors, Na\(^+\)/K\(^+\) ATPase (NKA) pump, Na\(^+\)/Ca\(^{2+}\) exchanger (NCX).

**RT-PCR** was used to determine mRNA values for MAS, NCX, SERCA, and AT2R.

### Discussion

* Protein expression for AT\(_R\), AT\(_R\) and Ang1.7-mediated-MAS receptors were significantly reduced in the cardiomyocytes of (mRen2)27.
* The protein expression of the NKA isoforms in the left ventricular myocytes show a two-fold increase in (mRen2)27.
* mRNA expression for MAS shows a significant decrease in (mRen2)27.
* mRNA profile for NCX reveals a twofold increase.
* Further, there is a decrease in the sarco/endo-plasmatic reticulum Ca\(^{2+}\)-ATPase (SERCA) in mRNA.

### Conclusions

* The relevant role of NKA and NCX in Na\(^+\) homeostasis was hypothesized that an increase in NKA isoforms (c1 & c2) would suggest a surge in the NCX exchanger to maintain Na\(^+\) influx in cardiomyocytes.
* The decrease in SERCA slow twitch would suggest that, in long term hypertension, Ca\(^{2+}\) is remaining in the cytoplasm causing increased contractility in cardiomyocytes.
* The data suggests that the optimum role of NKA, NCX and SERCA in handling of Na\(^+\) and Ca\(^{2+}\) in ventricular myocytes is different in RAAS-induced hypertension.

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