Expression of Na^+/K^+ ATPase Isoforms and Na^+/Ca^{2+} Exchanger Activity in Cardiac Myocytes of Ren-2 Transgenic model of Hypertension

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Abstract

Introduction: The Renin Angiotensin Aldosterone System (RAAS) is composed of various reactions in the regulation of blood pressure and plays crucial roles in cardiovascular physiology and pathophysiology. Classically, renin cleaves liver-derived angiotensinogen (AGT) into angiotensin I (Ang I), a decapeptide [Ang (1–10)] which is then further processed by angiotensin-converting enzyme (ACE) into the octapeptide Ang II [Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, Ang (1–8)]. Ang II is known to physiologically regulate blood pressure and is a key player in hypertension. Ang II causes vasoconstriction and production of aldosterone leading to retention of sodium (Na^+) and water resulting in cardiovascular dysfunction. **Objective:** is to study Ang II receptor mediation and the expression of intramembranous transporter of Na^+ and Ca^{2+} in cardiomyocytes and correlate activities of different Na^+/K^+-ATPase isoforms with the (mRen2)27 transgenic phenotype. **Methods:** The left ventricle of 12-15-week-old (mRen2)27 transgenic and Hannover-Sprague Dawley (HnSD) rats was isolated where protein was used for SDS PAGE and Western blotting analysis. **Results:** showed a significant increase in the Mean Arterial BP in (mRen2)27 transgenic rats but no change in pulse rate compared to HnSD control. There was a significant protein expression for Ang II receptor sub-type 1 (AT_1R) in (mRen2)27 when compared to control normotensive rodent. There were no differences in Na^+/K^+ ATPase α-1 isoform in both strains but a significant diminution of α-2 isoform in (mRen2)27 transgenic hypertensive rodents, suggesting an increase in intracellular Na^+ and Ca^{2+} concentrations in cardiac myocytes through the Na-Ca exchanger system. **Conclusion:** The finding suggests an increased AT_1 receptor protein, a diminished Na^+/K^+-ATPase α-2 isoform expression and augmented intracellular Na^+ and Ca^{2+} concentrations in (mRen2)27 transgenic hypertensive rodents, all of which may contribute to an increase in cardiac contractility, cardiac output, and sustained blood pressure.

Introduction

- Transgenic rat germline, (mRen2)27, expresses the mouse renin *Ren-2*^{d} gene and develops fulminant hypertension with a modest increase in plasma levels of active renin and angiotensin peptide.
- Ren-2 rodent represents a model of hypertension in which the genetic basis for the disease is known but the mechanism responsible for elevated blood pressure remains elusive. However, the model is thought to be mediated via extrarenal Renin-Angiotensin-Aldosterone system (RAAS).
- Studies have shown that there is increase in cardiac contractility and sympathetic nerve transmission mediated by AT_1 receptors and correlates with blood pressure.
- Since the ionotropic (contractility) activities of the cardiac myocytes are dependent on intracellular ATP-dependent-Na^+/K^+-pump and Na^+/Ca^{2+} exchanger system, **the objective here is to study the expression and behavior of intramembranous transporter of Na^+ and Ca^{2+} of the cardiac muscle and correlate the activities of different isoforms with the phenotype.**

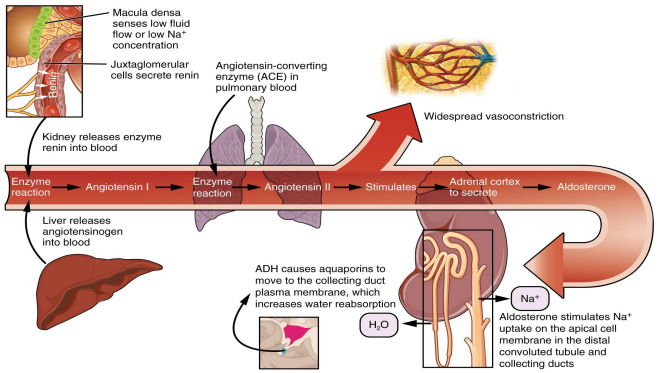
Methods

(a) Isolation of left ventricular muscle from the HnSD and m(Ren2)27,

(b) Protein isolation from the HnSD and m(Ren2)27 heart

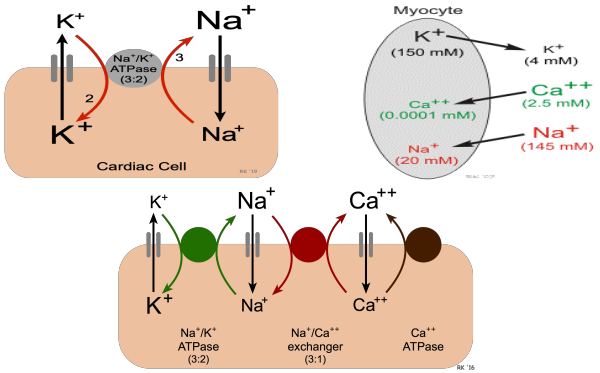
(c) SDS PAGE and Western blotting analysis of Na^+/K^+ -ATPase isoforms (α-1 & α-2), Na^+/Ca^{2+} activities and protein expression.

Scheme



Scheme: Renin Angiotensin Aldosterone System (RAAS) pathway – This mechanism is composed of various reactions that all aid in the regulation and deregulation of blood pressure.

Na^+/K^+ -ATPase, Na^+/Ca^{2+} Exchanger



Scheme: Na+/K+-ATPase, Ca2+ Exchanger – Calcium is removed from cells by two basic mechanisms:1) an **ATP-dependent Ca^{2+} pump** that actively removes calcium from the cell; 2) the **sodium-calcium exchanger**. During ventricular systole when the myocytes are depolarized, Ca^{2+} enters the cell through this exchanger. In contrast, during ventricular diastole when the cells are repolarized, Ca^{2+} leaves the cell through this exchanger. An increase in intracellular sodium concentration leads to an increase in intracellular calcium concentration through this exchange. This has important physiological implications including when the activity of the Na+/K+ -ATPase is decreased.

Results

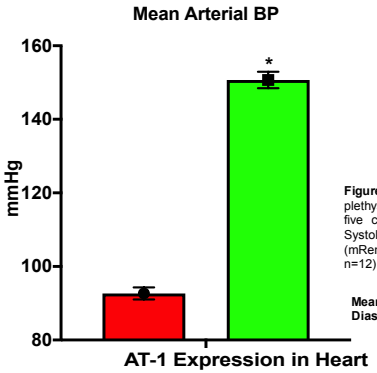


Figure 1: Blood Pressure Measurements. Tail-cuff plethysmography was used to record blood pressure for five consecutive days in 12 – 15 weeks old Rodents. Systolic and Mean arterial BP was significantly higher in (mRen2)27 rats when compared to HnSD rats (*p<0.0001; n=12). There was no change in the pulse rate.

Mean Arterial Blood Pressure (MAP) = Diastolic P + 1/3 Pulse Pressure

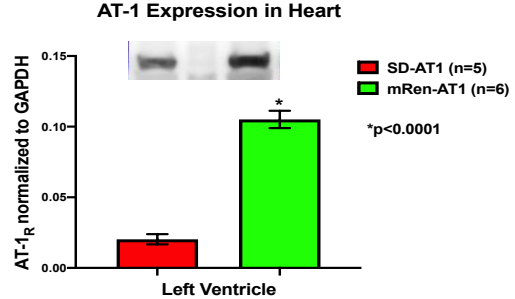


Figure 2: Angiotensin Type I (AT_1) Receptor Assessment via Western Blot analysis: There was a significant increase in the amount of AT_1R in (mRen2)27 as compared to HnSD at 12-15 weeks old. The mediation of the ventricular contractility is thought to be via AT_1R in (mRen2)27 compared to HnSD. *p<0.001; n stands for total number left ventricular muscle tissues used in the analysis in each group.

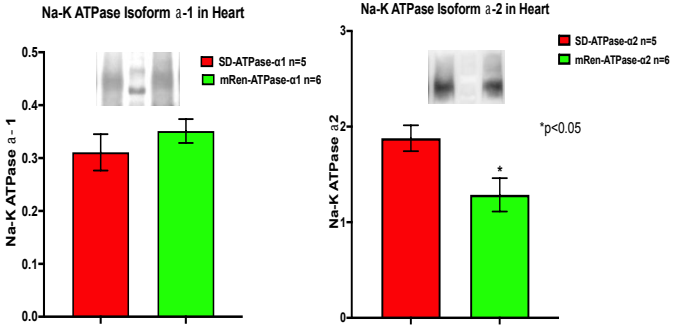


Figure 3: Na^+/K^+ -ATPase isoforms in the ventricular myocytes. There was no difference ATPase α-1 isoform in both strains but a significant decrease in α-2 isoform in (mRen2)27 transgenic rodents compared to HnSD, suggesting an increase in intracellular Na^+ and Ca^{2+} concentrations through the Na-Ca exchanger. *p<0.05; n stands for total number left ventricular muscles used in the analysis in each group.

Discussion

- The main objective is to identify the pathways directly responsible for the increase in contractility of the heart and hypertension in genetic form of hypertension, using the (mRen2)27 transgenic rat model.
- The AT_1R sub-type is significantly present in hypertension compared to control, HnSD, suggesting a possible source Ang II receptor mediation of the increased cardiac contractility.
- There was no change in the expression of ATPase α-1 isoform in (mRen2)27 and HnSD, but the ATPase α-2 isoform was significantly lowered in (mRen2)27 suggesting that the Na^+/K^+-ATPase pump activity is diminished, leading to an increase in intracellular Na^+ as well a possible increase in Ca^{2+} concentrations. The latter is due to the reversal of sodium-calcium exchanger system and a consequence in membrane depolarization that then leads to increased cardiac contractility and cardiac output as well as high blood pressure.
- This research is a part of a larger goal to seek the intracellular targets for genetic form of hypertension for therapeutic purposes.

Conclusion

- Blood pressure progressively increased significantly in (mRen2)27 animals to a maximum MAP of ~160 mmHg with no change in heart rate when compared to HnSD animals
- There was a significant increase in AT_1 receptor expression in myocytes of (mRen2)27 indicating that the ionotropic action of the ventricular muscle is mediated via AT_1 receptor subtype.
- The significant decrease in ATPase α-2 isoform in (mRen2)27 transgenic rodents compared to HnSD, suggests an increase in intracellular Na^+ and Ca^{2+} concentrations through the Na-Ca exchanger. This may contribute to cardiac contractility and hypertension in (mRen2)27 transgenic rodents.

Reference

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