Renin-Angiotensin-Aldosterone System on Cardiomyocyte and the Impact of Anti-Hypertensive Drug on Myocardial Excitability

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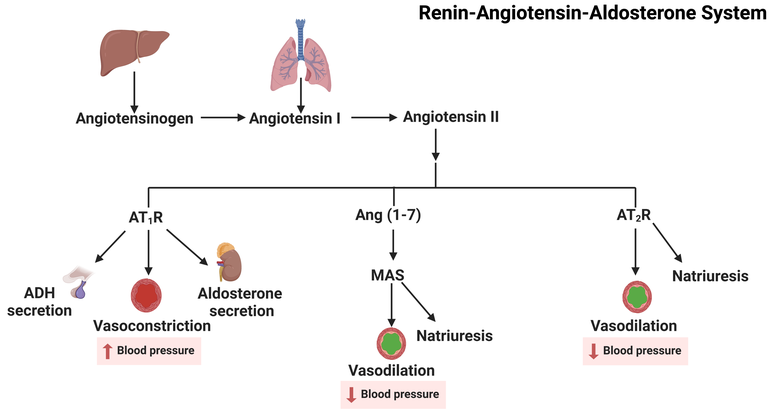
Abstract

Hypertension is a polygenic condition that characterizes one of the most common and relevant cardiovascular complications that contributes to approximately 690,000 deaths per year in the United States. Renin-angiotensin-aldosterone system (RAAS) plays a significant role in systemic and neurogenic hypertension [1, 2]. Angiotensin II (Ang II), an octapeptide, is a key hormonal peptide that interacts with AT_1 and AT_2 G-protein-coupled receptors (GPCR). The effects of AT_1R mediate excitatory responses, and AT_2 serves as the protective arm of RAAS at the level of cardiomyocytes. Angiotensin-converting enzyme-2 (ACE2) converts AngII into Ang-(1-7) mediated by MAS receptor, a GPCR for Ang-(1-7), to mitigate myocardial excitation and regulate blood pressure. Angiotensin 1-7 (Ang 1-7) has been shown to reduce blood pressure [3], cardiac contractility [4, 5], and reactive oxygen species (ROS) [6], mediated via MAS1 proto-oncogene protein (MAS). The objective is to study the expression of RAAS receptors on ventricular myocytes isolated from hypertensive rodents to assess the implication of the AngII in the presence or absence of antihypertensive drugs. There was a correlation in the circulating plasma AngII with blood pressure in hypertensive rodents. The blood pressure was reversed with angiotensin receptor blocker (ARB) suggesting the impact of RAAS. Cardiac expression for AT_1R showed a significant increase compared to the control group ($p<0.001$) but was abolished in the treated group with angiotensin receptor blocker (16mg/kg candesartan) and a vasodilator (10mg/kg di-hydralazine). Conversely, AT_2R expression in hypertensive group was significantly lowered compared to control group ($p<0.001$). However, the antihypertensive drug treatments did not reverse AT_2 response to control level suggesting a non-reversal of AT_2R expression when blood pressure is returned to normal. Furthermore, there was a significant decrease in the protein expression of MAS receptors for angiotensin 1-7 (Ang1-7) peptides but was returned to control levels in the treatment group. These results suggest that while AT_1R mediates excitability and enhancement of cardiac contraction in hypertensive condition, MAS receptor mediates the metabolite of AngII, through Ang1-7 and, along with AT_2R to serve as the protective arm of angiotensin peptides. This work implies that changes in the expression of the receptors on the surface of the cells are a key mechanism involved in the signaling pathways that lead to the manifestation of hypertension. It also suggests that treatments for hypertension works by restoring receptor expression to basal level.

Introduction

- Hypertension, is a condition characterized by a systolic pressure of ≥140 mmHg or greater and diastolic pressure of ≥90 mmHg. Older age is a major factor that increases the odds of developing hypertension.
- Hypertension is a major risk factor for the development of stroke, heart attack, heart failure, kidney disease, and other cardiovascular complications.
- The cascade of physiologic events which lead to hypertension is not fully elucidated.
- However, treatment with angiotensin converting enzyme (ACE) inhibitors or AngII receptor antagonists is extremely efficient.
- Recently, the importance of AT_2R and MAS receptor in sympathetic nerve transmission has been speculated to attenuate ROS activities. It is speculated Ang1-7 would reduce ROS production.
- Thus, **the objective is to study the expression of RAAS receptors on ventricular myocytes isolated from (mRen2)27 transgenic hypertensive rodents to assess the implication of the AngII peptide in the presence of antihypertensive treatment.**



Methods

(a) Mouse *Ren2*^{d} gene inserted into genome of rat – m(Ren2)27 transgenic hypertensive model
(b) Plasma assay for AngII and Ang 1-7 before and after antihypertensive drug treatment
(c) Isolation of left ventricular cardiomyocytes before and after antihypertensive drug treatment
(d) Protein and RNA isolation from the HnSD and m(Ren2)27 cardiomyocytes
(e) SDS PAGE-Western blotting and qRT-PCR analysis of RAAS receptor protein expression.

Results

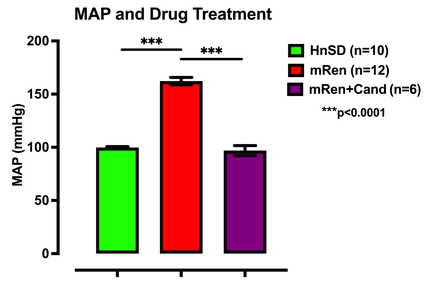


Figure 1 Mean Arterial blood pressure and antihypertensive drug treatment. Blood pressure of mRen rat is significantly higher compared to HnSD rats but a significant decrease in the presence of candesartan and dihydralazine treatments (n=6; p<0.0001).

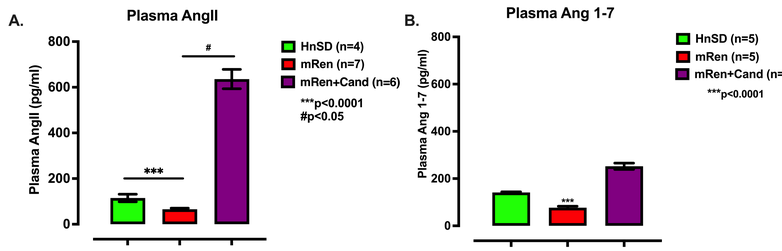


Figure 2 Plasma AngII from mRen compared to HnSD was significantly lowered ($p<0.05$) but significantly higher in mRen rodents treated with candesartan and dihydralazine ($p<0.0001$). There was also a significant increase in the treated vs control ($p<0.0001$). **Plasma Ang1-7** is significantly decreased in mRen compared to HnSD. Following the infusion of candesartan and dihydralazine drugs, the circulating Ang 1-7 rose above the HnSD level ($p<0.0001$).

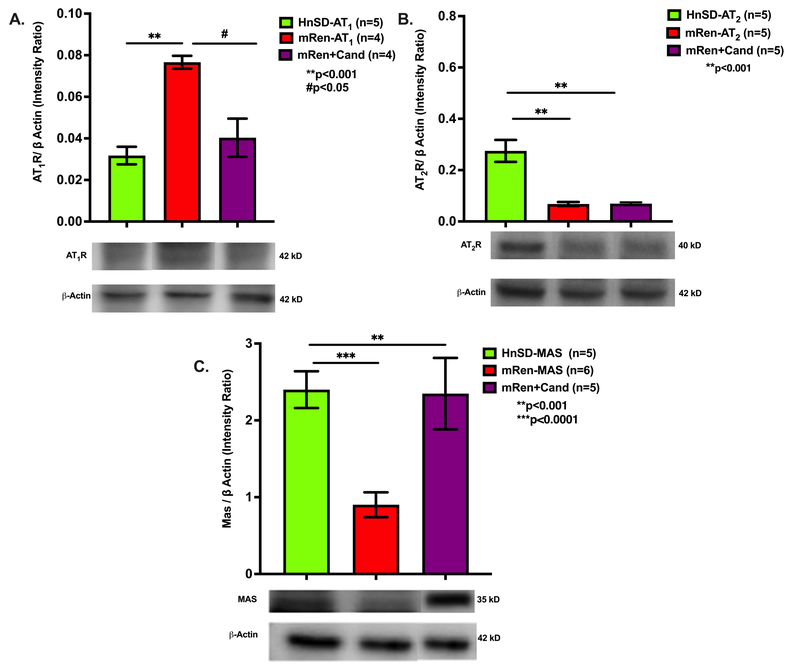


Figure 3 Western blotting protein expression of AT_1, AT_2 receptor subtypes, and Mas receptor specific protein density in the left ventricle (LV). Receptor density was measured by Western blot hybridization using specific antibodies in LV isolated from SD, mRen, and mRen antihypertensive drug treated rats.Top: Densitometry analyses of protein level normalized to β-actin. Image analyses of the signals are normalized to β-actin. Values in each panel are mean ± SEM; #p<0.05, **<0.001, and ***<0.0001 representative Western blot. Bottom: representative Western blot, respectively.

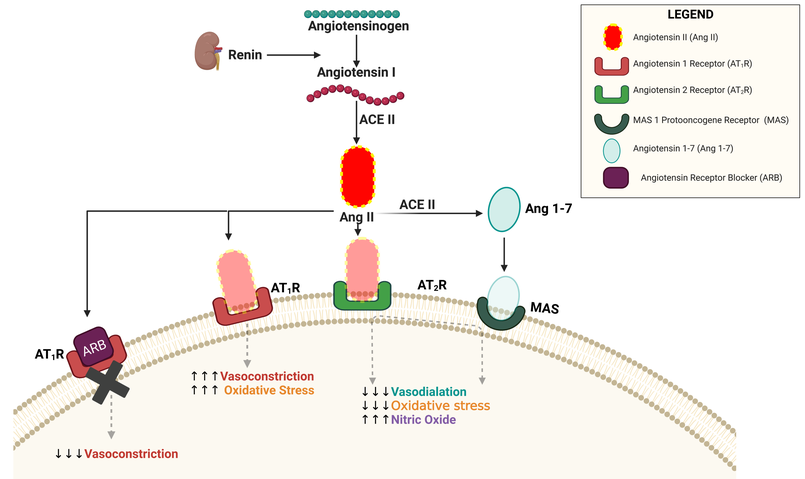


Figure 4 Angiotensin II pathway diagram and the mechanism of action for candesartan, which is an AT_1R blocker.

Conclusion

- Blood pressure in this model of hypertension was reduced to normal by candesartan and dihydralazine treatments suggesting that the angiotensin receptor blockers (ARBs) and vasodilators are effective in RAAS-mediated hypertension (**Figure 1**).
- There is a decrease in circulating AngII in the mRen animals compared to the HnSD, however the antihypertensive treated group showed a significant increase in plasma AngII suggesting ARBs–AngII competitive binding on AT_1 receptor (**Figure 2 A,B).**
- There is an increased protein expression for AT_1 receptor in hypertension but was abolished in the mRen rodents treated with anti-hypertensive drugs. This suggests a reversal of AT_1R protein expression when blood pressure was returned to normal (**Figure 3A**).
- Protein expression for AT_2 receptor showed a significant decrease in cardiomyocytes isolated from the mRen2 animals compared to HnSD. However, the anti-hypertensive drug did not restore the protein expression for AT_2 receptor (**Figure 3B).**
- There was a decreased MAS protein expression in the cardiomyocytes isolated from of mRen animals compared to HnSD. However, those treated with anti-hypertensive drug restored the MAS receptor expression which further suggests that the protective arm of RAAS protein expression (i.e., MAS) may restore when blood pressure is normalized (**Figure 3C).**
- Expression of the receptors on the cells are a key mechanism involved in the signaling pathways that lead to the manifestation of hypertension.

Reference

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