Research Article

Role of angiotensin II type 2 receptor during regression of cardiac hypertrophy in spontaneously hypertensive rats

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Abstract

We previously reported that the AT_1 receptor antagonist valsartan and the angiotensin converting enzyme (ACE) inhibitor enalapril decrease DNA synthesis and stimulate apoptosis in interstitial fibroblasts and epicardial mesothelial cells during regression of ventricular hypertrophy in spontaneously hypertensive rats (SHR). To examine the role of the AT_2 receptor in this model, we studied hearts from SHR treated with valsartan or enalapril either alone or combined with the AT_2 antagonist PD123319 for 1 or 2 weeks. Apoptosis was evaluated by quantification of DNA fragmentation or by TUNEL labeling. At 1 week, valsartan significantly increased ventricular DNA fragmentation, increased apoptosis in epicardial mesothelial cells, and decreased DNA synthesis. At 2 weeks, ventricular DNA content and cardiomyocyte cross-sectional area were significantly reduced. These valsartan-induced changes were attenuated by PD123319 co-administration. However, valsartan-induced increases in apoptosis of left ventricular interstitial non-cardiomyocytes was unaffected by the AT_2 blocker. Enalapril-induced changes were similar to those observed with valsartan but were not affected by co-treatment with PD123319. These results demonstrate that AT_1 and AT_2 receptors act in a coordinated yet cell-specific manner to regulate cell growth and apoptosis in the left ventricle of SHR during AT_1 receptor blockade but not ACE inhibition. J Am Soc Hypertens 2013;7(2):118–127. © 2013 American Society of Hypertension. All rights reserved.

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Introduction

Left ventricular hypertrophy is a major risk factor for heart disease and is a strong predictor of overall morbidity

and mortality.[1] We have previously demonstrated that inhibition of the renin angiotensin system (RAS) with either angiotensin converting enzyme (ACE) inhibitors or angiotensin type 1 (AT_1) receptor antagonists induces regression of left ventricular hypertrophy in adult spontaneously hypertensive rats (SHR) that is mediated in part by reduction in cardiomyocyte size and apoptosis of cardiac fibroblasts.[2] Specifically, we showed that treatment with a RAS inhibitor reversed left ventricular hyperplasia in SHR via a selective and transient induction of apoptosis in interstitial fibroblasts and epicardial cells[2]; an effect that is accompanied by a sustained inhibition of cardiac DNA synthesis.[3] In addition, in a model of accelerated fibrotic cardiac remodeling in SHR, AT_1 receptor antagonist-induced apoptosis of non-cardiomyocytes was associated with reductions in collagen deposition.[4]

Interestingly, fibroblast apoptosis occurs significantly earlier with AT_1 antagonists (at 1 week) than ACE

inhibitors (at 2 weeks), despite equivalent reduction of blood pressure.[5] These findings may suggest a distinct mechanism of apoptosis induction not merely due to a reduction in AT_1 receptor signaling. During AT_1 receptor antagonist treatment, angiotensin II (Ang II) would be free to act on unblocked AT_2 receptors.[6,7] AT_2 receptor stimulation has been suggested to be cardioprotective in some, though not all, models of heart disease[8–12] and has also been shown to counteract the AT_1 mediated growth effects by inducing apoptosis in certain cell types.[3,13] Therefore, we proposed that Ang II stimulation of AT_2 receptors may be involved in the relatively early apoptosis induction during AT_1 receptor blockade but not during ACE inhibition. Consistent with this, we have demonstrated that co-administration of the AT_2 receptor antagonist PD123319 prevents apoptosis induction, growth suppression, and vascular hypertrophy regression in the aorta of SHR treated with valsartan but not enalapril.[3] In order to examine the role of AT_2 receptors in cardiac remodeling during AT_1 receptor blockade or ACE inhibition, the hearts of SHR from this previous study[3] were examined.

Methods

Animal Procedures

The 9- to 10-week-old male SHR in this study were previously randomized to the different treatment groups and used to investigate the aortic responses to angiotensin pathway blockers.[3] At the time of sacrifice, in addition to harvesting the aortas for studying the effects of RAS inhibition on vascular smooth muscle cell (VSMC) apoptosis,[3] the hearts were also excised and used in the present study to investigate the effects of these treatments on cardiac apoptosis. Briefly, SHR purchased from Charles-River (St. Constant, Quebec, Canada) were housed for at least 10 days before initiation of study. Food and water were administered ad libitum. Rats (n = 5–8 per group) were treated for 1 or 2 weeks with the selective AT_1 receptor antagonist valsartan (30 mg/kg per day orally; gift of Novartis, Toronto, Ontario, Canada), the selective AT_2 receptor antagonist PD123319 (30 mg/kg per day; gift of Parke-Davis, Ann Arbor, MI) via osmotic minipump (Alzet, model 4ML1 or 4ML2; Alza, Palo Alto, CA), or a combination of both drugs. A subgroup of rats received enalapril (30 mg/kg per day orally, Sigma Chemicals, St. Louis, MO) alone or in combination with PD123319 for 2 weeks. Controls received vehicle. All experimental procedures were approved by the Université de Montréal ethics committee on animal care and performed in accordance with the guidelines of the Canadian Council on Animal Care.

Systolic blood pressure was determined in conscious restrained rats by the tail-cuff method as we described previously.[14] Hearts were excised, both ventricles were isolated together, weighed, and an equatorial cross-section (3 mm) was fixed overnight in 4% paraformaldehyde and later paraffin-embedded for in situ apoptosis detection and for hypertrophy and hyperplasia measurements. The remaining tissue was prepared for DNA synthesis (in vivo [^{3}H]-thymidine incorporation into DNA during the last 2 hours before sacrifice), oligonucleosomal DNA fragmentation (indicative of apoptosis),[7] and cardiac AT_1 and AT_2 receptor gene quantifications as described previously.[3]

Analysis of Apoptosis

Our previous time course studies showed that DNA fragmentation is maximally increased in the SHR heart after 1 week of AT_1 antagonist treatment and 2 weeks of ACE inhibitor treatment during regression of hypertrophy.[2,5] These time points for valsartan (1 week) and enalapril (2 weeks) were selected for the present study.

To assess the cell type undergoing apoptosis, tdt-mediated dUTP-FITC or AMCA nick end labeling (TUNEL) was performed as previously described.[2] TRITC-α-sarcomeric actin immunoreactivity and propidium iodide staining were used as markers for cardiomyocytes and total nuclei respectively, while TRITC- lectin reactivity was employed as a marker for endothelial cells. Figure 1 shows representative photomicrographs of TUNEL and immunohistological staining. Image analysis of fluorochrome-labeled cells was performed in a blinded manner in fluorescence microscopy using appropriate filters. Quantification was conducted separately in the right and left ventricles. All TUNEL-positive cell type-specific nuclei (cardiomyocyte, non-cardiomyocyte: endothelial and fibroblast) in each section were counted, and results are expressed as the percentage of TUNEL-positive cell type-specific nuclei per cross-section as we described previously.[2]

Hypertrophy and Hyperplasia Measurements

Using the National Institutes of Health Image J program, 50 random measurements of transversely oriented cardiomyocyte cross-sectional areas were collected in order to establish hypertrophy values. Moreover, the total number of cardiomyocytes within each ventricle of SHR from the 2-week valsartan study was stereologically assessed as per Anversa et al.[15] A similar procedure was used to calculate total non-cardiomyocytes as previously described.[2]

Determination of AT_2 Receptor Protein Expression Levels

In left ventricles, AT_2 receptor protein expression was determined via immunoblotting. The epicardial layer was delicately separated from remaining tissue to assess regional AT_2 receptor expression via western blot. Protein was extracted and separated via SDS-PAGE. Receptor