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CHRONIC ANGIOTENSIN RECEPTOR BLOCKADE SUPPRESSES INTRACARDIAC ANGIOTENSIN II IN ANG II-INFUSED RATS

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Abstract

Accumulation of angiotensin II (Ang II) in tissues is an Ang II-receptor mediated process. In pigs, acute Ang II receptor blockade (ARB) reduced the heart-to-plasma ratio of Ang II following acute infusion. However in rats, chronic ARB treatment increased heart Ang II levels suggesting that a differential response to ARB treatment may exist in the mammalian heart. Furthermore, the changes in heart Ang II receptors following chronic ARB treatment are not well described. To address the discrepancy in heart Ang II levels following ARB treatment, 3 groups (n=6) of rats were chronically studied: 1) control, 2) angiotensin II (80 ng/min for 28 d) and 3) angiotensin II + olmesartan (ARB; 10 mg/kg/d for 21 d). Ang II-infusion increased intracardiac Ang II by 40% (53 ± 2 vs. 74 ± 6 fmol/g) and intrarenal Ang II over 2-fold (96 ± 6 vs. 207 ± 14 fmol/g), and chronic ARB treatment decreased Ang II by 48% in the heart (50 ± 7 fmol/g) and over 2-fold in the kidney (92 ± 7 fmol/g) suggesting that accumulation of Ang II in the heart is receptor-mediated as in the kidney. Ang II increased plasma aldosterone 2.5-fold (1.4 ± 0.1 vs 3.5 ± 1.2 nM) and was exacerbated by ARB treatment (5.6 ± 1.0 nM). Intracardiac aldosterone was exacerbated by ARB treatment (control: 2.2 ± 0.3 ; Ang II: 2.7 ± 1.1 ; ARB: 7.8 ± 1.7 pmol/g). Suppression of intracardiac Ang II with ARB is consistent with the existing view of Ang II receptor mediated uptake by tissues.

Keywords

aldosterone breakthrough; angiotensin receptors; hypertension; RAS

Introduction

The accumulation of renal angiotensin II (Ang II) is mediated primarily by an angiotensin receptor type 1 (AT1)-mediated process. (1–5) However, Zou et al.(1) report that chronic (2 wks) infusion of Ang II did not increase the tissue levels of Ang II in the heart or adrenals. Furthermore, chronic treatment with the angiotensin receptor blocker (ARB) losartan in the Ang II-infused rat increased intracardiac Ang II 4-fold and intra-adrenal Ang II by 80% suggesting that the process of receptor-mediated sequestration of Ang II may be tissue specific. Conversely, in pigs acute (10 min) treatment with an ARB (L-158, 809) resulted in about a 10-fold decrease in heart-to-plasma ratio of acutely (15 min) infused radiolabeled Ang II suggesting that, at least acutely, the accumulation of Ang II in the heart is receptor mediated.(3) Thus, the data on the accumulation of Ang II in the heart following either acute

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Conflict of Interest

Authors have no conflicts to disclose.

or chronic Ang II infusion are incongruous and warrant further evaluation to clarify the inconsistency of the response to ARB treatment.

As a component of the renin-angiotensin system, elevated Ang II stimulates aldosterone secretion resulting in elevated plasma aldosterone. Because aldosterone has been reported to increase Ang II receptor (i.e. AT1) number and binding *in vitro*, (10, 14, 15) a potential mechanism by which Ang II infusion results in accumulation of tissue Ang II can be through a plasma aldosterone-dependent increase in Ang II receptor number and binding. Therefore, in theory, ARB treatment can contribute to decreasing tissue Ang II levels (in addition to displacing Ang II) by reducing plasma aldosterone, which decreases AT1 number and binding, and thus, tissue Ang II levels. However, the relationships among aldosterone and Ang II levels in the heart in Ang II-dependent hypertension are not well described.

The present study elucidated the effects of chronic ARB treatment on intracardiac Ang II and aldosterone content in Ang II-infused rats. We hypothesized that chronic ARB treatment decreases intracardiac Ang II, similar to that observed in the kidney, associated with a decrease in heart aldosterone.

Methods

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee's of both Kagawa Medical University and the University of California, Merced in accordance to the guidelines for the care and use of animals established by these institutions.

Animals and Procedures

Male Sprague-Dawley rats (Clea Japan Inc., Tokyo, Japan) approximately 200–225g were randomly assigned to three experimental groups (n=6/group): 1) control, 2) angiotensin II (Ang II; 80 ng/min) and 3) angiotensin II + olmesartan (ARB; 10 mg/kg/d in diet; Daiichi-Sankyo Co. Ltd., Tokyo). Control and Ang II rats were maintained on a normal rat chow diet (Daiichi-Sankyo Co. Ltd., Tokyo). The dosage of ARB is consistent with that used previously to successfully block Ang II. (16, 17) Food consumption rates in this study were measured weekly to verify treatment goals were met. Systolic blood pressure (SBP) was monitored daily by radiotelemetry implanted in the animals and used to evaluate the effectiveness of the Ang II infusion and ARB. (18, 19) Animals were anesthetized with sevoflurane prior to implanting a biotelemetric transducer (PA-C40, DSI, St. Paul, MN) subcutaneously in the dorsal flank with the catheter lead extending inguinally and into the femoral artery and into the abdominal aorta. The animals were allowed to recover for 7 days from the effects of the surgery. The animals were again anesthetized with sevoflurane and implanted with an osmotic mini-pump (Durect Corp, Cupertino, CA) subcutaneously to infuse Ang II (80 ng/min; Phoenix Pharmaceuticals, Belmont, CA) for 28 days. Each animal was maintained individually in metabolic cages in a temperature and light controlled room. Throughout the experiment, animals had unrestricted access to water and their specific diet. Ang II infusion began on day 1 of the study and the animals were allowed 7 days to become hypertensive prior to treatment to mimic the conditions in which ARB is prescribed. The ARB diet began on day 7 for a period of 21 days. On the last day of the study, 24-hr urine voids were recorded and an aliquot was collected for analysis of total protein. Urine samples were diluted 1:100 prior to measurement of total protein by photospectrometry (BioRad, Hercules, CA). Urinary total protein excretion (U_{TPV}) was calculated as the product of urine volume and urinary total protein concentration.

Dissections

On day 28, animals were weighed, decapitated, and trunk blood collected into chilled vials containing 5 mM EDTA plus protease inhibitor cocktail (PIC; Sigma, St. Louis, MO) for the measurement of plasma aldosterone, Ang II and plasma renin activity (PRA). The heart was removed patted dry and weighed. Sections were placed in 10 ml of cold methanol and homogenized for Ang II extractions or 10 ml of PBS and homogenized for aldosterone extraction. The left kidney was removed, cleaned, weighed, and placed in a glass vial containing 10 ml of methanol, and homogenized for extraction of Ang II. The right kidney was removed, cleaned, weighed, flash-frozen in liquid nitrogen and stored at -80°C for future analysis. The right adrenal was removed, cleaned, weighed, placed in a glass vial containing 10 ml of cold PBS, and homogenized for extraction of aldosterone.

Hormone Analyses

PRA was measured using a commercially available RIA kit (DiaSorin, Stillwater, MN). Adrenal and heart aldosterone were extracted from their homogenates with ethyl acetate. Heart, adrenal and plasma aldosterone concentrations were measured by a commercially available RIA kit (DPC, Los Angeles, CA). Heart and kidney homogenates and plasma (1.0 ml) were extracted for Ang II measurements with details of the Ang II extraction and assay procedures described previously.(20)

Statistics

Data are presented as means $\pm$ SE. Plasma, organ, and densitometry measurements were compared by one-way analysis of variance (ANOVA) and linear regression model. For all cases, if significance ($p < 0.05$) was detected, a Fisher's Protected Least Significant Difference test was applied post hoc. Statistics were performed using Statview software (SAS, Cary, NC).

Results

Blood Pressure, and Body and Heart Mass

The infusion of Ang II increased mean SBP 46% ($174 \pm 10^*$ mm Hg) above control (119 ± 7 mm Hg) and ARB treatment was effective in reducing the pressure to control levels ($125 \pm 6^{\#}$ mm Hg) by day 28 demonstrating the effectiveness of both the Ang II infusion and ARB treatment (Figure 1). By day 28, mean body mass was 11% lower than control in the Ang II group, and ARB treatment had recovered mean body mass to 8% of control, which was not significantly different. Absolute heart mass was greater in both Ang II and ARB groups above control (Table 1). Relative heart mass in the Ang II group was 46% greater than control and 14% greater than ARB treated rats suggesting that blockade of Ang II receptor had partially alleviated the hypertrophy (Table 1).

Intra-organ Angiotensin II

Ang II infusion increased intracardiac Ang II by 40% (53.1 ± 2.1 vs. 74.0 ± 6.3 fmol/g) from control (Figure 2). Treatment with ARB decreased mean intracardiac Ang II by 32% (50 ± 7 fmol/g), which is similar to control levels. Infusion of Ang II increased mean intrarenal Ang II over 2-fold (96 ± 6 vs. 207 ± 13 fmol/g) and ARB treatment (92 ± 7 fmol/g) returned levels to control (Table 2).

Intra-organ Aldosterone

Intracardiac aldosterone was not significantly increased after Ang II infusion (2.2 ± 0.3 vs. 2.7 ± 1.0 pmol/g); however, treatment with ARB increased mean intracardiac aldosterone nearly 4-fold (7.8 ± 1.7 pmol/g) compared to control (Figure 3). Ang II infusion increased

mean adrenal aldosterone by 54%, and treatment with ARB exacerbated levels, inducing over a 3-fold increase in mean adrenal aldosterone compared to control (Table 2).

Plasma Hormones

Infusion of Ang II in both the untreated and ARB treated groups suppressed plasma rennin activity (PRA) compared to control (Table 2). Plasma Ang II levels were not different between control and Ang II-infused groups; however, following treatment with ARB there was a 2-fold increase (Table 2). Infusion of Ang II induced a 2.5-fold increase in mean plasma aldosterone and ARB treatment increased levels an additional 60% from Ang II (Table 2).

Urinary Total Protein Excretion

After 28 days, Ang II infusion increased ($p < 0.01$) U_{TPV} nearly 2.5-fold (27 ± 3.5 mg/d) from control (11 ± 1.8 mg/d) and ARB had no further effect (28 ± 4.7).

Discussion

Accumulation of angiotensin II in kidneys has been shown to be a receptor mediated process; (1–3, 5, 21, 22) however there is some discrepancy in the response of the heart to elevated Ang II during acute and chronic Ang II infusion and between acute and chronic ARB treatment. While chronic infusion of Ang II was previously shown to increase intra-renal Ang II levels 2-fold, simultaneous measures of Ang II levels in the heart or adrenals were not affected with infusion suggesting that the accumulation of Ang II is tissue specific. (1) In the same study, ARB treatment in the Ang II-infused rat completely ameliorated the intra-renal Ang II levels; however, the heart and adrenal Ang II levels were increased 4-fold and 80%, respectively, further suggesting that the accumulation of Ang II in extra-renal tissues is not a receptor-mediated process. (1) Conversely, acute ARB treatment reduced the heart-to-plasma ratio of radiolabeled Ang II nearly 10-fold in the acutely infused pig heart suggesting that the accumulation of Ang II in the mammalian heart is angiotensin receptor mediated as in the kidney. (3) In accordance with our hypothesis, Ang II levels in the heart in the present study increased during chronic infusion and were completely ameliorated with chronic ARB treatment suggesting that the accumulation of Ang II in the heart is an AT1 receptor-mediated process similar to that observed in the kidney.

The increase of plasma Ang II levels following ARB treatment and in the presence of reduced PRA suggests that increased plasma Ang II levels are the consequence of reduced tissue sequestration and increased displacement from their receptors, and substantiates the idea that regulation of intracardiac Ang II levels is AT1-mediated during Ang II infusion. Furthermore, the complete amelioration of intra-renal Ang II (and SBP) following ARB treatment in the present study provides evidence of the effectiveness of the blockade of the angiotensin receptor, (1, 23) and suggests that the blockade of angiotensin receptors was systemic and not just localized in the heart. Interestingly, despite the robust blockade of the angiotensin receptors, ARB treatment failed to recover the Ang II-induced suppression of PRA. While ARB treatment in Ang II-infused Sprague-Dawley rats has been shown to recover the Ang II-induced suppression of PRA (1, 24) an important and significant difference between this and those previous studies is the timing of the initiation of ARB. In the present study, ARB (olmesartan) was not provided until after the first week of Ang II infusion when the animals were hypertensive (approximately 74% of maximal SBP), whereas in the previous studies (1, 24) ARB (losartan or candesartan) was provided simultaneously at the onset of the study when animals are still normotensive and PRA is not suppressed. In normotensive subjects with suppressed PRA (< 0.65 ng Ang I/ml/hr), various ARBs (including olmesartan and valsartan) failed to recover PRA levels within 24 hr

following an acute dosage (25) suggesting that ARB treatment is not effective at recovering PRA levels once they are maximally suppressed (as may be the case with Ang II infusion), and alludes to a potential change in sensitivity of the JGA to Ang II. This phenomenon clearly warrants further investigation.

Another important and novel discovery of the present study is the increase in heart aldosterone levels following chronic Ang II receptor blockade. In the present study, chronic ARB treatment was associated with a nearly 4-fold increase in intracardiac aldosterone. In addition, chronic ARB treatment in the present study did not prevent adrenal aldosterone production, thus the exacerbated levels of plasma and adrenal aldosterone are consistent with the phenomenon of aldosterone “breakthrough” or “escape”. (15, 32, 33) Treatment with ARB failed to reduce aldosterone production and secretion and instead were associated with a “breakthrough” or “escape” event in the present study suggesting that aldosterone escape is independent of AT1 mediation and likely the result of compensatory changes to chronic blockade of adrenal angiotensin receptors. While aldosterone breakthrough has been reported,(32) to the best of our knowledge this is the first report of local aldosterone breakthrough in the heart following RAS inhibition. Thus, both local and systemic aldosterone breakthrough events occurred simultaneously in the present study suggesting that diagnosis of systemic aldosterone breakthrough in patients may be associated with local breakthrough in the heart necessitating co-therapy with a mineralocorticoid receptor antagonist. The similarity in the levels of aldosterone between plasma and heart following ARB treatment provides evidence for effective sequestration of circulating aldosterone by the heart in the presence of inappropriately elevated plasma aldosterone, and would argue against the notion of any significant aldosterone production by the heart.(34) A limitation of the present study is the lack of data that address the mechanisms by which ARB induced a breakthrough event. Elucidation of the cellular mechanisms of aldosterone escape warrant further investigation, especially if it can be determined that elevated tissue levels associated with escape are detrimental.

It is well established that the progression of renal disease is associated with increased proteinuria. A review of the clinical data suggests that ARB treatment reduces proteinuria but the results are variable.(35) Data regarding the effectiveness of ARB treatment on alleviating the proteinuria in Ang II-dependent hypertensive models is scarce. Olmesartan (ARB) has been shown to effectively alleviate the proteinuria after 14 days in Ang II infused rats.(36) However, in the present study a similar dose of olmesartan failed to alleviate proteinuria in Ang II infused rats after 3 weeks of treatment. Because inappropriately elevated aldosterone contributes to the progression of renal injury as indicated by increased proteinuria,(37) the discrepancies in these results may be attributed to differences in aldosterone levels, which unfortunately were not reported in the previous study. (35) Thus, the exacerbation of aldosterone by ARB treatment in the present study likely impaired the ability of the ARB to alleviate the proteinuria.

Perspectives

The present results suggest that chronic treatment with an ARB promotes aldosterone escape locally at the level of the heart despite the benefit of reducing arterial blood pressure. Therefore, patients on chronic ARB treatment may be more susceptible to aldosterone-induced cardiovascular injury if the aldosterone is unabated. Further studies are needed to confirm that the elevated levels of intracardiac aldosterone are physiologically relevant and functional. Future studies that focus on mineralocorticoid receptor regulation in the heart during disruption of RAS is needed to confirm whether or not local aldosterone breakthrough has the potential to be physiologically relevant. Alternatively, increased intracardiac aldosterone may induce intracellular events that are

independent of MR activation that would require interventions to inhibit the intracellular signaling of those mechanisms.

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Statement of Author Contributions and Acknowledgements

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. D Conte, J Viscarra and RM Ortiz conducted the experiments. A Nishiyama provided animals, accommodations and reagents. D Conte and RM Ortiz wrote the manuscript. The authors would like to thank Drs. F. Yuan, N. Pelisch and K. Asato, and Mr. A. Mamalis for their excellent technical assistance. We would like to thank Drs. J. Funder and C. Gomez-Sanchez for their much-appreciated input on this study, their insightful discussion on this topic and critical review of an earlier draft of this manuscript.

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Figure 1. Mean ($\pm$ SE) systolic blood pressure ($n=6$) in Control, Angiotensin II (Ang II) and Ang II + angiotensin receptor blocker (ARB)-treated animals. The arrow indicates when the ARB treatment was induced (day 7) with a diet containing olmesartan (10 mg/kg/d). * Denotes significant ($p<0.05$) difference from Control.

Figure 2.

Mean ($\pm$ SE) heart angiotensin II (Ang II) content from Control, Angiotensin II (Ang II) and Ang II + angiotensin receptor blocker (ARB)-treated animals. Ang II infusion (80 ng/min $\times$ 4 wk) was initiated on day 1 and treatment with ARB (10 mg/kg/d $\times$ 3 wk) was initiated on day 7. * Denotes significant ($p < 0.05$) difference from Control. # Denotes significant ($p < 0.05$) difference from Ang II.

Figure 3.

Mean ($\pm$ SE) heart aldosterone content from Control, Angiotensin II (Ang II) and Ang II + angiotensin receptor blocker (ARB)-treated animals. Ang II infusion (80 ng/min $\times$ 4 wk) was initiated on day 1 and treatment with ARB (10 mg/kg/d $\times$ 3 wk) was initiated on day 7.

* Denotes significant ($p < 0.05$) difference from Control.

Table 1

Mean ($\pm$ SE) body mass, heart mass, and relative heart mass from Control, Angiotensin II (Ang II), and Ang II + angiotensin receptor blocker (ARB)-treated rats after 28 days.

	Control	Ang II	Ang II + ARB
Body Mass (g)	400 $\pm$ 14	355 $\pm$ 10 *	370 $\pm$ 12
Heart Mass (g)	1.12 $\pm$ 0.06	1.46 $\pm$ 0.06 *	1.33 $\pm$ 0.06 *
Relative Heart Mass (g/ 100 g BM)	0.28 $\pm$ 0.01	0.41 $\pm$ 0.02 *	0.36 $\pm$ 0.01 *,#

* Significantly ($p < 0.05$) different than control,

significantly ($p < 0.05$) different than Ang II group.

Table 2

Mean ($\pm$ SE) plasma renin activity (PRA), angiotensin II, aldosterone and tissue hormone levels after 28 days of Ang II-induced hypertension and 21 days of ARB treatment.

	Control	Ang II	Ang II + ARB
PRA (ng Ang I/L/h)	4.4 $\pm$ 1.0	0.6 $\pm$ 0.2*	0.2 $\pm$ 0.1*
Plasma Angiotensin II (fmol/mL)	32 $\pm$ 4	38 $\pm$ 3	70 $\pm$ 7*,#
Plasma Aldosterone (nM)	1.4 $\pm$ 0.1	3.5 $\pm$ 1.2*	5.6 $\pm$ 1.0*,#
Adrenal Aldosterone (pmol/mg of tissue)	235 $\pm$ 50	363 $\pm$ 94*	755 $\pm$ 169*,#
Kidney Ang II (fmol/g of tissue)	96 $\pm$ 6	207 $\pm$ 14*	92 $\pm$ 7 [#]

* Significantly ($p < 0.05$) different than control,

significantly ($p < 0.05$) different than Ang II.