Angiotensin Subtype-2 Receptors Inhibit Renin Biosynthesis and Angiotensin II Formation

Helmy M. Siragy, Chun Xue, Peter Abadir, Robert M. Carey

Abstract—Renin is regulated by angiotensin subtype 1 (AT1) receptor, but it is unknown whether angiotensin subtype 2 (AT2) receptor contributes to this regulation. We hypothesized that AT2 receptors inhibit angiotensin II (Ang II) through inhibition of renin biosynthesis. We monitored changes in renal Ang II, renin mRNA and protein expression, and plasma renin concentration (PRC) in response to renal cortical administration of the AT1 receptor blocker valsartan or the AT2 receptor blocker PD 123319 (PD) in conscious rats administered low sodium intake (LS). Renal interstitial Ang II increased by 47-fold in response to LS and increased further in response to valsartan or PD by 67-fold and 61-fold from normal sodium diet (NS) and by \(\approx 41\%\) and 29\% from LS, respectively. Renin mRNA increased 63\% during LS, and either valsartan or PD increased it further by 600\% and 250\% from NS and 538\% and 187\% from LS, respectively. Similarly, renal renin content and PRC increased in response to LS and increased further in response to combined LS and valsartan or PD administration. Immunostaining for renal renin protein demonstrated an increase in its expression in juxtaglomerular and tubular cells during LS and increased further during AT1, or AT2, receptor blockade. These data demonstrate for the first time to our knowledge that AT2 receptors regulate the renin-angiotensin system activity via inhibition of renin synthesis. (Hypertension. 2005;45:1-5.)

Key Words: receptors, angiotensin II ■ renin ■ kidney ■ angiotensin

Angiotensin II (Ang II) is the major effector hormone of the renin-angiotensin system (RAS). Most of the known physiological functions and pathologic effects associated with Ang II are mediated by the angiotensin subtype 1 (AT1) receptor, including renin inhibition.\(^1\) In contrast, the functions of the angiotensin subtype 2 (AT2) receptor are still being elucidated. The AT2 receptor is expressed abundantly during fetal development and declines after birth.\(^1-3\) In mammalian adults, the AT2 receptor has been reported in multiple organs, including the kidney.\(^4-6\) Currently, the AT2 receptor has several described functions related to inhibition of cell growth, promotion of cell differentiation, and stimulation of apoptosis.\(^7-12\) Previously, we demonstrated that during sodium depletion, the AT2 receptor mediates renal production of bradykinin, nitric oxide (NO), and cGMP.\(^13-17\) Unlike the well-known effect of AT1 receptor on renin production,\(^18-19\) the influence of the AT2 receptor on renal renin biosynthesis and Ang II production is not known.

In this study, we tested the hypothesis that the AT2 receptor inhibits renal renin synthesis and Ang II production. We used multiple techniques including renal interstitial microdialysis,\(^13-17\) real-time quantitative reverse-transcription polymerase chain reaction, immunohistochemistry, and enzyme-linked immunosassays for monitoring changes in renal Ang II, renin mRNA, renin protein expression, and plasma renin concentration to investigate whether intrarenal AT2 receptors regulate renin production and Ang II formation. We monitored renal interstitial levels of Ang II in conscious rats during sodium restriction, a condition known to increase AT2 receptor expression, and during AT1 and AT2 receptor blockade.\(^5\) A distinct advantage of the microdialysis technique\(^13\) is the ability to monitor renal Ang II levels in conscious rats without undesirable hemodynamic changes. In this study, we demonstrate for the first time to our knowledge that AT2 receptors regulate the activity of the RAS through inhibition of renin biosynthesis in young rats.

Methods

Renal Microdialysis Technique

For the determination of renal interstitial fluid (RIF) Ang II, we constructed a microdialysis probe as previously described.\(^13-17\) In vitro, best recovery for Ang II was observed with a perfusion rate of 3 \(\mu\text{L/min}\), and was \(\approx 53\%\). A negligible amount of Ang II sticks to the polyethylene tubes of the dialysis probes, as demonstrated by the in vitro recovery of \(^{125}\text{I}\)Ang II at 99.8\%.\(^15-20\)

Animal Preparation

The experiments were approved by the University of Virginia Animal Research Committee and conducted in accordance with institutional guidelines. Experiments were conducted in 4-week-old female Sprague–Dawley rats (n=8 in each group; Harlan Teklad, Madison, Wis). For collecting RIF, the microdialysis probe was inserted in the kidney as previously described.\(^13-17\) For infusions into the renal cortical interstitial space, a 10-cm-long polyethylene tube
(PE 60; Becton Dickinson, Sparks, Md) was inserted into the right renal cortex as previously described. The interstitial infusion rate was determined from preliminary studies and is optimum at 5 μL/min (a rate that ensures distribution of the infused substance to the entire renal cortex without dislodging the catheter from the kidney). To demonstrate renal distribution of interstitially infused substances, using florescence microscopy we were able to localize fluorescence-labeled Ang II in the renal cortex. Rats were housed under controlled conditions (temperature: 21 ± 1°C; humidity: 60 ± 10%; lighting 8 to 20 hours) and allowed 7 days to recover and to acclimatize to the laboratory. Experiments were started at the same time (8:00 AM) each day to avoid any diurnal variation of the measured substances. During the first and last 30 minutes of each study, systolic arterial pressure was measured every 10 minutes in the rat tail (Rat Tail Manometer-Tachometer System, Natsume model KN-210; Peninsula Laboratories, Inc, Belmont, Calif) and the recorded values were averaged for each study.

Effects of Sodium Depletion and Angiotensin AT1 or AT2 Receptor Blockade on RIF Ang II
In this study, rats (n=8 each group) were placed in metabolic cages. Baseline 24-hour urine collections were obtained for calculation of urinary sodium excretion, and RIF samples were obtained for Ang II while rats were consuming a normal sodium diet (0.28% NaCl; BioServe Biotechnologies, Frenchtown, NJ). Then, rats were placed on a low-sodium diet (0.05% NaCl) for 8 days. At day 7, we monitored 24-hour urinary sodium excretion (U NaCl). While the rats continued to consume the low-sodium diet (day 8), RIF Ang II levels were monitored during intrarenal cortical interstitial administration (5 μL/min for 8 hours) in random order of D5W vehicle (n=8), PD 123319 (n=8; Parke-Davis-Warner Lambert Co, Ann Arbor, Mich), and methods described previously. Negative controls included omission of primary and secondary antibodies.

The aforementioned studies were repeated in another group of animals during normal sodium intake. Renal renin mRNA, renin protein expression, protein expression and total renin concentration were monitored in response to AT1, or AT2 receptor blockade.

Renin Immunohistochemistry
Kidney tissues were fixed in Bouin solution and embedded in paraffin. Six-micrometer sections were stained for renin using a previously characterized polyclonal goat anti-rat renin antibody (a gift from Dr Tadashi Inagami at the Vanderbilt University), avidin-biotin-peroxidase (Vestacast Inc ABC kit; Vector Laboratories, Burlingame, Calif), and methods described previously. Negative controls were considered statistically significant.

Results
Systolic Blood Pressure Responses to Low Sodium Intake Alone and Combined With Intrarenal Administration of Valsartan of PD
During normal sodium intake, systolic blood pressure was 590 ± 34 μmol/dl and increased to 357 ± 7 μmol/dl (P<0.0001) after 7 days of low sodium intake. Systolic blood pressure (n=8 each group) in rats on normal sodium intake was 108 ± 1 mm Hg, and did not change significantly during low-sodium diet alone or combined with intrarenal cortical administration of valsartan or PD.

RIF Ang II Responses to Low Sodium Intake
RIF Ang II levels (Figure 1A) during normal sodium intake were 2±0.2 fmol/mL (n=8) and increased during dietary sodium restriction to 96±1.5 fmol/mL (P<0.00001). Intrarenal cortical administration of valsartan or PD (n=8 each treatment) during low sodium intake caused a further increase in RIF Ang II to 136±2 and 124±2 fmol/mL, respectively (P<0.00001 from normal sodium and P<0.0001 from low sodium intake).

Total Renal Ang II Content in Response to Low Sodium Intake Alone and Combined With Intrarenal Administration of Valsartan or PD
During normal sodium intake (n=8), total renal Ang II content was 164±16 fmol/g kidney weight (Figure 1B). Low sodium intake (n=8) increased renal Ang II content to 338±49 fmol/g kidney weight (P<0.0001). Intrarenal corti-
Renal renin mRNA and Total Renin Content Response to Low Sodium Intake Alone and Combined With Intrarenal Administration of Valsartan or PD

During normal sodium intake, the levels of the renal renin mRNA and total renin content were very low and did not change in response to valsartan or PD treatment (data not shown). Renal renin mRNA (n = 8) increased significantly by \( \approx 80\% \) \( (P<0.0001) \) in response to low sodium intake (Figure 2A). Intrarenal cortical administration of valsartan (n = 8) or PD (n = 8) during low sodium intake caused further increase in renal renin mRNA (620% and 250%, respectively) compared with normal \( (P<0.0001) \) and low \( (P<0.0001) \) sodium intake. During normal sodium intake, total renal renin content (Figure 2B) was 30\( \pm \)5 \( \mu \)g/kg kidney tissue \( (n=8) \) and increased to 150\( \pm \)3 \( \mu \)g/kg kidney tissue per hour \( (P<0.0001) \) in response to low sodium intake \( (n=8) \). Intrarenal cortical administration of valsartan \( (n=8) \) or PD \( (n=8) \) during low sodium intake caused a further increase \( (313\pm10 \) and \( 344\pm12 \) \( \mu \)g/kg kidney weight, respectively) in total renal renin content \( (P<0.0001) \) compared with normal or low sodium intake.

Renal Renin Immunostaining in Response to Low Sodium Intake Alone and Combined With Intrarenal Administration of Valsartan or PD

There was no renin staining when IgG preimmune was used as control (Figure 3A and 3F). During normal sodium intake (Figure 3B and 3G), renin immunostaining was very low, localized mainly in the juxtaglomerular apparatus, and did not change in response to valsartan or PD treatment (data not shown). Low sodium intake (Figure 3C and 3H) caused significant increase in renin immunostaining in the glomeruli and juxtaglomerular apparatus. Combined low salt intake with valsartan (Figure 3D and 3I) or PD (Figure 3E and 3J) caused further increase in renin staining in glomeruli, juxtaglomerular apparatus, and tubules compared with normal (Figure 3B and 3G) and low salt (Figure 3C and 3H) intake alone.

Plasma Renin Concentration in Response to Low Sodium Intake Alone and Combined With Intrarenal Administration of Valsartan or PD

During normal sodium intake, plasma renin concentration levels (Figure 4) were 10\( \pm \)1.1 ng/mL and increased in response to low sodium intake to 25\( \pm \)4.2 ng/mL \( (P<0.01) \). Plasma renin concentration increased further to 62\( \pm \)12.2 and 48\( \pm \)11.4 ng/mL during combined low sodium intake and valsartan or PD treatment, respectively \( (P<0.0001) \) from normal sodium intake and \( P<0.01 \) from low sodium intake.

Discussion

This study demonstrates, for the first time to our knowledge, the regulation of the RAS by the AT\(_2\) receptor. We hypothesized that AT\(_2\) receptor decreases generation of Ang II through reduction in renin synthesis. Our data support this hypothesis based on the observed increase in renal renin mRNA, renin protein, and Ang II during AT\(_2\) receptor....
blockade. The renin and Ang II response to the AT2 receptor blockade was similar to the response observed during valsartan treatment and suggests that both AT1 and AT2 receptors have an inhibitory effect on the activity of the RAS. This result was unexpected because previous studies7,8,10,15,28 demonstrated opposite effects for these 2 receptors. AT2 receptor inhibition of renin synthesis and secretion is in agreement with its presumed vasodilator and cardiovascular protective effects.15,17 It is highly unlikely that the observed results are caused by the interaction of PD with the AT1 receptor because the infusion rate used was small, one-fifth of previously reported doses,13–17 and did not produce any changes in blood pressure. Based on the affinity studies, PD at the dose used in this study is specific for the AT2 receptor and does not interact with the AT1 receptor.29

All the components of the RAS are present within the kidney and local renal production of Ang II has been demonstrated.20 Renin is the rate-limiting enzyme in the synthesis of Ang II.30 In addition to juxtaglomerular cells, renin is produced by mesangial19 and tubular32 cells. Angiotensinogen and angiotensin-converting enzyme are generated within the kidney.30 AT1 and AT2 receptors are also located within the kidney.28 In particular, AT2 receptors are present in juxtaglomerular cells and have been shown to inhibit prorenin processing.33

Previously published data demonstrated a short negative feedback loop of the AT1 receptor to suppress renin and Ang II production.1,18,19 A regulatory role for the AT2 receptor in modulating RAS activity has not been described previously. A murine strain with disruption of the AT2 receptor gene displays slightly elevated baseline blood pressure, exaggerated vasopressor response to Ang II, increased prostaglandin E2 (PGE2), and reduced NO and cGMP production.16 In those studies, systemic or renal tissue renin or Ang II levels were not evaluated. In a renal vascular hypertension rat model,15 AT2 receptor blockade caused further increase in blood pressure. Taken together, current and previous data suggest that the AT2 receptor regulates the RAS either directly or through multiple mechanisms involving NO, cGMP, or PGE2.

The increase in Ang II in parallel to the increase in renin mRNA, renin protein, and plasma renin concentration suggests that the AT2 receptor regulates the RAS mainly through increased production of renin. The current study cannot differentiate whether the increase in the activity of the RAS is directly linked to decreased AT2 receptor activity or is directly linked to reduction in its mediator NO and cGMP. The influence of NO is controversial, with studies showing stimulation,34,35 inhibition,16 or no direct influence37–39 on renin production. Cyclic GMP has been shown to inhibit renin expression and secretion.40 Activation of cGMP-dependent protein kinases by cGMP decreased renin secretion from the isolated perfused rat kidney, isolated renal juxtaglomerular cells, and kidney slices.40–42 PGE2 enhances renin production43 through stimulation of EP3 and EP4 receptors. Absence of AT2 receptor activity increases renal PGE2 levels through enhancing the activity of AT1 receptors13,16 or decreasing the metabolism of PGE2 to 6-ketoPGF1α.44 Thus, our previous finding of AT2 receptor mediation of NO,14 cGMP,13 and PGE213,16 production supports conclusion that this receptor regulation of renin production, at least in young animals.

Absence of PD effects on renin synthesis and secretion during normal sodium intake, a condition associated with low AT2 receptor expression,5 strengthens the argument for its involvement in renin regulation. Previous studies demonstrated that AT1 and AT2 receptors counterbalance each other.28 The current study demonstrates a rare instance in which both of these receptors share a similar function.

**Perspectives**

This study introduces the novel concept of AT2 receptor regulation of the RAS activity. Development of an AT2
receptor agonist could help provide a pharmacological tool for further RAS inhibition, in addition to angiotensin-converting enzyme and AT1 receptor blockers, to manage cardiovascular disease.

Acknowledgments

This study was supported by grant HL-57503 and DK-61400 from the National Institutes of Health to Helmy M. Siragy, MD. H.S. was the recipient of Research Career Development Award K04-HL-03006 from the National Institutes of Health.

References


Angiotensin Subtype-2 Receptors Inhibit Renin Biosynthesis and Angiotensin II Formation
Helmy M. Siragy, Chun Xue, Peter Abadir and Robert M. Carey

Hypertension. published online November 8, 2004;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2004/11/08/01.HYP.0000149105.75125.2a.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/