Paradoxical Regulation of Short Promoter Human Renin Transgene by Angiotensin II

Henry L. Keen, Curt D. Sigmund

Abstract—We previously reported the generation of transgenic mice containing the entire human renin gene with a 900-bp promoter. To determine whether all the required elements for angiotensin II–mediated suppression of human renin are present in these mice, angiotensin II was chronically infused by means of osmotic minipump at both low and high doses, 200 and 1000 ng/kg per minute, respectively. Blood pressure was measured by tail-cuff, and kidney renin mRNA levels were quantitated using ribonuclease protection assays. Blood pressure was unchanged in mice receiving either vehicle or low-dose angiotensin II infusion but was increased by approximately 40 mm Hg with the higher dose of angiotensin II. Mouse renin mRNA decreased by >60% during both pressor and nonpressor angiotensin II infusion. Human renin mRNA was not suppressed by nonpressor angiotensin II and was paradoxically increased 1.9-fold by pressor angiotensin II. The lack of upregulation during nonpressor angiotensin II suggested that the increase might be pressure-mediated. To test this, the angiotensin II-induced increase in blood pressure was prevented by coadministration of the vasodilator, hydralazine (15 mg/kg per day). Hydralazine alone decreased blood pressure (−27±3 mm Hg) and increased mouse renin mRNA 2.4-fold. Human renin mRNA was unresponsive to this vasodilator-induced fall in pressure and despite the normalization of blood pressure by hydralazine, high-dose angiotensin II still caused a 2.1-fold increase in human renin mRNA. Thus, the first 900 bp of the human renin promoter does not contain all the elements required for appropriate angiotensin II–mediated suppression of human renin mRNA. (Hypertension. 2001;37[part 2]:403-407.)

Key Words: angiotensin II ■ renin ■ transgenic mouse ■ hypertension ■ blood pressure

Angiotensin II (Ang II) is one of the body’s most powerful regulators of sodium balance and blood pressure. For example, during sodium deprivation, Ang II–induced increases in sodium reabsorption and vascular resistance restore sodium balance while maintaining blood pressure at a near-normal level. Ang II plays a role in the etiology of cardiovascular disease, because reduction in circulating and tissue Ang II levels by administration of ACE inhibitors has been associated with decreased incidence of stroke, heart failure, myocardial infarction, and other cardiovascular complications. A similar beneficial action has been reported in initial studies using specific Ang II receptor antagonists. In humans, secretion of the aspartyl protease renin by interacting with secondary factors such as prostaglandins. This interaction might be direct or possibly through an influence on the macula densa–mediated tubuloglomerular feedback mechanism. Lastly, it has been suggested that a change in physical forces in the afferent arteriole itself (ie, blood pressure, stretch) can directly regulate renin.

We previously reported the generation and characterization of transgenic mice containing the entire human renin gene, including a 896-bp promoter sequence. Because of the species specificity of the reaction between renin and angiotensinogen, these single transgenic mice were phenotypically normal. Although there was variable nonrenal renin expression in different transgenic lines, the predominant site of expression was localized to JG cells, and no expression was observed in any other cell type in the kidney. The transgene was appropriately upregulated by β-adrenergic receptor stimulation and by ACE inhibition. However, in double transgenic mice containing both human genes (renin and angiotensinogen), human renin mRNA was significantly elevated. This response was opposite to that of mouse renin mRNA, and was surprising because the chronically increased Ang II levels and blood pressure in the double transgenic mice...
should have suppressed human renin. The purpose of the present study is to further examine this paradoxical upregulation of human renin mRNA.

Methods

Animal Care
All surgical procedures and care of the mice were conducted in accordance with National Institutes of Health guidelines, using protocols approved by the Animal Care and Use Committee of the University of Iowa. Mice were fed standard mouse chow (Teklad) and received water ad libitum.

Experimental Protocols
Systolic blood pressure was determined by tail-cuff (Visitech Systems BP-2000) after a 7- to 10-day training period during which the mice were acclimated to the tail-cuff procedure. Baseline blood pressure measurements are the average result over a 5-day control period. On the last day of the baseline period, mice were anesthetized with metamizol and metoxic minipumps (Alzet, model 1007D) were implanted subcutaneously. Isotonic saline served as the vehicle for all infusions. Ang II (Sigma) was infused at both a subpressor and a pressor dose, 200 and 1000 ng/kg/min, respectively. A peripheral vasodilator, hydralazine (Sigma), was infused at 15 mg/kg per day, either alone, to significantly lower blood pressure, or in conjunction with the pressor dose of Ang II to normalize blood pressure. Experimental blood pressure data represents the average value over the last 3 days of the 5-day experimental period. At the end of the experiment, mice were killed by CO2 asphyxiation and kidneys were immediately frozen on dry ice. RNA was isolated from kidney samples by homogenization in guanidinium isothiocyanate, using a modification of the method described, and was stored at −80°C.

RNase Protection Assay
A commercially available RNase protection assay kit (Ambion) was used to determine the mRNA levels for mouse renin (MREN) and human renin (HREN). A total of 10 μg of total RNA was used for each assay. Probe templates for HREN and MREN were partial cDNA sequences amplified by RT-PCR and cloned into pBluescript (Stratagene). Mouse actin (ACT) cDNA template was obtained from commercial software (ImageQuant, Molecular Dynamics). Values for mRNA abundance are arbitrary values normalized to ACT, the internal control for all samples.

Statistical Analysis
All data are presented as mean±SEM. Between-group comparisons were made with one-way analysis of variance (ANOVA) using commercial software (SigmaStat). Bonferroni t-tests were used for post hoc analysis when a statistical difference was observed. Probability value less than 0.05 represent statistical significance.

Results
To eliminate the potentially confounding influence of lifetime hypertension on the renin response, studies were conducted in normotensive single transgenic mice, and the duration of treatment was limited to 5 days. Ang II was infused at both a low and a high dose to determine whether the response is dose dependent. In addition, because the higher dose of Ang II infusion increases both blood pressure and Ang II levels, blood pressure was normalized by concurrent vasodilator infusion in a separate group of mice to separate the effect of high Ang II versus that of high blood pressure.

Renal Renin Expression During Increased Angiotensin II
Before minipump implantation, baseline blood pressure averaged 111±4, 102±4, and 111±4 mm Hg in mice designated to the untreated saline, and subpressor and pressor Ang II infusion groups, respectively. Although in mice infused with subpressor Ang II (200 ng/kg per minute), there was a tendency for blood pressure to increase, there was no statistical difference compared with saline infused mice (115±6 versus 112±6 mm Hg) (Figure 1). Higher dose infusion of Ang II (1000 ng/kg per minute) caused a significant and sustained increase in blood pressure (156±5 vs 15 mm Hg). Typical RNase protection analysis for mouse and human renin mRNA are shown in Figure 2A and 2B, respectively. MREN mRNA was significantly decreased in both low-dose (22±2% of vehicle) and high-dose (33±5% of vehicle) Ang II–infused mice (Figure 2C). In contrast, HREN mRNA was not decreased by subpressor Ang II (98±26% of vehicle) and was paradoxically increased by pressor Ang II (187±12% of vehicle, Figure 2C).

Figure 1. Systolic blood pressure measured by tail cuff in transgenic mice receiving a 5-day subcutaneous infusion of low-dose (LA, hatched bars, n=4) or high-dose (HA, black bars, n=5) Ang II, 200 or 1000 ng/kg/min, respectively. Isotonic saline infusion (S, open bar, n=4) served as vehicle. *Significant difference from saline-treated group (P<0.05).

Figure 2. Transgenic mice received a 5-day subcutaneous infusion of low-dose (LA) or high-dose (HA) Ang II, 200 or 1000 ng/kg/min, respectively. Isotonic saline infusion (S) served as vehicle. (A) Representative RNase protection assay of mouse renin (REN) mRNA response. (B) Representative RNase protection assay of human renin (REN) mRNA response. (C) Quantitated data from RNase protection assays during low (LA, hatched bars, n=4) or high (HA, black bars, n=5) Ang II infusion or during saline (S, clear bars, n=4) infusion. Values for mRNA abundance are arbitrary values normalized to mouse actin (ACT), the internal control for all samples. *Significant difference from saline-treated group (P<0.05).
Role of Blood Pressure in Mediating the Paradoxical Increase in 900-HREN

Before minipump implantation, baseline blood pressure averaged 103 ± 2, 100 ± 4, and 105 ± 2 mm Hg in mice designated to the untreated saline, hydralazine and hydralazine plus pressor Ang II infusion groups, respectively. Hydralazine alone decreased blood pressure to 73 ± 1 mm Hg, and in combination with pressor Ang II, normalized the blood pressure level to a value (107 ± 3 mm Hg) not different than that in saline-infused mice (104 ± 4 mm Hg) (Figure 3). Associated with the decreased blood pressure in hydralazine mice was an increase in MREN mRNA (248 ± 62% of vehicle, Figure 4A and 4C). HREN mRNA was unresponsive to the hydralazine-induced fall in blood pressure and, despite the normalization of blood pressure by hydralazine; pressor Ang II resulted in an increase in HREN mRNA (211 ± 27% of vehicle, Figure 4B and 4C). Hydralazine did not affect the MREN mRNA response (36 ± 3% of vehicle) to pressor Ang II.

Discussion

The major finding of the present study is that Ang II–mediated suppression of the HREN gene in transgenic mice requires DNA sequences outside the first 900 bp of the HREN promoter. Moreover, sequences necessary for the HREN gene to appropriately respond to pharmacologically induced changes in blood pressure may be missing from the transgene used herein. This finding confirms and extends our previous finding that 900-HREN responds appropriately to some physiological stimuli but not to others.11

The ability to express human genes in the mouse has provided an excellent opportunity to study in vivo the molecular mechanisms regulating gene expression. With respect to HREN, the species specificity of the reaction between renin and angiotensinogen is particularly advantageous. Because human renin cannot cleave mouse angiotensinogen, single transgenic mice containing only human renin are phenotypically normal. Recent studies both from our laboratory and others have demonstrated that the HREN gene is appropriately regulated in transgenic mice in response to multiple physiological cues when genomic constructs containing very large amounts of flanking DNA (35 kb) are used.14,15 This provides strong evidence that all the trans-acting factors needed for HREN regulation are present in the mouse. However, when smaller amounts of flanking DNA are used, as in the present study, the regulatory response is appropriate only for certain stimuli.

This finding suggests that distinct regions of HREN flanking DNA are responsible for mediating the response to the different intracellular signaling pathways regulating renin. The second messenger cAMP is a potent stimulator of renin, and much evidence suggests that β-adrenergic stimulation of renin is mediated by a cAMP-dependent mechanism.16 We previously reported that isoproterenol, a β-adrenergic agonist, appropriately stimulated HREN in 900-HREN mice, suggesting that the first 900 bp of the HREN promoter contains those DNA elements needed to respond to cAMP.11 In support of this, a functional cAMP response element (CRE) exists within the 900-bp promoter (position −225 to −218) and reporter constructs containing a 900-bp HREN promoter respond appropriately to cAMP in vitro.17

Another potent stimulus of renin synthesis is ACE inhibition. Because ACE inhibition reduces the level of circulating Ang II, some investigators have postulated that the primary stimulus for increased renin is the withdrawal of a direct suppressive action of Ang II on JG cells. Angiotensin type 1 (AT 1) receptors have been identified on JG cells, and in vitro, Ang II has been shown to suppress renin release from rat kidney slices.6,18 However, recent data from gene-targeted mice has cast doubt on this direct action of Ang II, by suggesting that a direct action of Ang II on JG cells is not required for the normal compensatory upregulation of renin mRNA during ACE inhibition (or AT 1A receptor blockade).7
ACE inhibition is associated not only with a reduction in circulating Ang II but also with a significant fall in blood pressure. Decreased renal perfusion pressure per se is a potent stimulus for enhanced renin synthesis and probably plays some role in the renin response to ACE inhibition. During chronic vasodilator infusion in the present study, blood pressure was reduced to a level similar to that during ACE inhibition and expression of the mouse renin gene was significantly increased, apparently secondary to the blood pressure fall, although a pressure-independent action cannot be excluded. In contrast, 900-HREN was unresponsive to the pressure fall, suggesting that the appropriate upregulation of 900-HREN during ACE inhibition is probably not due to reduced blood pressure, but perhaps to mechanisms that remain unclear.

Recent evidence has implicated an important role for prostaglandins in the renin response to ACE inhibition. The inducible isomerase of cyclooxygenase (COX-2), a key step in the enzymatic cascade leading to production of prostaglandins, has been localized to the juxtaglomerular area. Cheng et al have reported that renal cortical COX-2 expression is upregulated by chronic ACE inhibition in rats, and that the increase in renin is markedly attenuated by treatment with a COX-2 specific inhibitor. In vitro studies have demonstrated that prostaglandin E2 (PGE2) is capable of stimulating renin by binding to the EP4 receptor subtype on JG cells and activating the cAMP pathway. One possibility is that increased renin during ACE inhibition is due, at least in part, to PGE2-induced activation of the cAMP pathway. If so, the appropriate upregulation of 900-HREN during ACE inhibition would be consistent with the possibility that 900-HREN is cAMP responsive.

Ang II is a negative regulator of renin; however, results from our studies of 900-HREN transgenic mice suggest the possibility that the molecular pathways that stimulate renin under the conditions of Ang II (ie, ACE inhibition) and those that suppress renin during increased Ang II may be distinct. The failure of Ang II to suppress 900-HREN suggests the possibility that some regulatory elements mediating the negative response to Ang II (ie, repressor) are not present in the first 900 bp of the HREN promoter. However, even if the failure to suppress HREN was due to the lack of important regulatory sequences, it is still difficult to explain why 900-HREN was paradoxically upregulated by pressor Ang II. Because this upregulation was not observed in mice during subpressor Ang II, one possibility we considered was that the upregulation was pressure mediated. However, prevention of the increased blood pressure during high-dose Ang II infusion by concurrent administration of a peripheral vasodilator had no effect on the renin mRNA response. These data combined with the failure to upregulate 900-HREN during vasodilator-only infusion suggest that 900-HREN may be unresponsive to any changes in blood pressure, at least under the conditions tested. Recent reports have demonstrated that the pressure-mediated action on renin depends on the calcium-calmodulin pathway, suggesting such an element may be further upstream of the HREN gene.

By what other mechanisms can Ang II paradoxically increase the 900-HREN transgene? One possibility is an interaction between Ang II and the cGMP pathway that has been reported to have both a positive and negative influence on renin. Two known activators of this pathway are nitric oxide and atrial natriuretic peptide, both of which have been reported to be stimulated by Ang II. A recent study in knockout mice provided evidence that the inhibitory influence of the cGMP pathway involves activation of cGMP kinase type II. The stimulatory effect depends on the cAMP pathway and involves cGMP inhibition of cAMP phosphodiesterase isofrom 3. It is possible that the elements responding to transcription factors modulated by cGMP kinase may lie upstream of the HREN promoter used in the 900-HREN transgene. If so, the net effect of Ang II activation of the cGMP pathway might be to stimulate renin.

Another potential candidate sequence is an enhancer of renin transcription that lies close to the MREN promoter (−2.6 kb) but far upstream of the HREN promoter (−12 kb). Future studies will be required to fully elucidate the pathways activated by Ang II and to determine the DNA regulatory regions transducing those responses.

Acknowledgments

Funds to support this work were provided by the National Institutes of Health (NIH), HL48058 and HL55006 to C.D.S. H.L.K. is the recipient of a NIH National Research Service Award (HL10044). Transgenic mice were generated and maintained at the University of Iowa Transgenic Animal Facility, which is supported in part by the College of Medicine and the Diabetes and Endocrinology Research Center. We sincerely thank Norma Sinclair, Lucinda Robbins, and Trish Lovell for their outstanding technical assistance regarding the generation and characterization of transgenic mice, Xiaojie Zhang for assistance with RNase protection assays, and Deborah Davis for assistance with blood pressure recordings.

References


Paradoxical Regulation of Short Promoter Human Renin Transgene by Angiotensin II
Henley L. Keen and Curt D. Sigmund

Hypertension. 2001;37:403-407
doi: 10.1161/01.HYP.37.2.403

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 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/37/2/403

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/