

Hypertension

7

THE YEAR IN
HYPERTENSION

RAYMOND TOWNSEND

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Contents

Editor and contributors vii

- 1.** Basic science in hypertension 1
Carlos M Ferrario, Jewell A Jessup, Aaron J Trask, Jasmina Varagic
- 2.** Biomarker update: current and future roles 19
Carmine Savoia, Ernesto Schiffrin
- 3.** Blood pressure measurement: is there more to this than systolic, diastolic and pulse pressures? 39
Adrian Covic, Paul Gusbeth-Tatomir, David Goldsmith
- 4.** Clinical trials in high-risk hypertension: implications of small differences in blood pressure 59
Gordon McInnes
- 5.** Controversies in hypertension 75
William Elliott
- 6.** Hypertension and diabetes 95
Atul Chugh, George Bakris
- 7.** Hypertension: emerging therapies 109
Domenic Sica
- 8.** Hypertension and pregnancy 127
Jason G Umans
- 9.** The role of proteinuria in management of hypertension 145
Tazeen Jafar
- 10.** Hypertension: epidemiology, management, ethnicity and sociology 165
Raymond Townsend
- 11.** Renovascular disease and secondary hypertension 191
Stephen Textor
- 12.** Systolic and pulse pressure in hypertension: what have we learnt? 211
Michel E Safar, et al.

-
- 13.** Recent transplant issues in hypertension 227
Daniel Salzberg, Matthew Weir
- 14.** Nutrition and obesity studies for the management of
hypertension and reducing cardiovascular risk 243
Holly Kramer, Richard Cooper
- 15.** Future prospects for management of hypertension 263
Norman Kaplan
- Acronyms/abbreviations* 285
- Index of papers reviewed* 287
- General index* 297

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1

Basic science in hypertension

CARLOS M FERRARIO, JEWELL A JESSUP, AARON J TRASK,
JASMINA VARAGIC

Introduction

The renin–angiotensin system (RAS) executes critical functions in both short- and long-term maintenance of homeostatic arterial blood pressure. Angiotensin II (Ang-II), the most potently active peptide of the system, exhibits a vast array of acute and chronic effects, which contribute to the maintenance of blood pressure under normal physiological conditions and which, under stressed conditions such as dehydration or hemorrhage, redeem the pressure deficit. In the scenario of hypertension, however, these same effects of Ang-II can have deleterious ramifications that contribute to cardiovascular pathology. The modern tools of molecular biology and the availability of drugs that prevent either the catalytic activity of angiotensin-forming enzymes or the binding of the biologically active angiotensin peptides [i.e. Ang-II and angiotensin-(1–7)] to its receptors have shed new light into the mechanisms by which the system functions both as a circulating and as a local tissue regulator of cellular function and overall homeostasis (Fig. 1.1). From both a biochemical and functional approach, the RAS should be viewed today as both an endocrine and a local tissue hormonal system. The *endocrine* system, in which changes in renin secretion from the kidney regulate the formation of Ang-II in the blood, functions to make rapid adjustments in arterial pressure and circulating blood volume by initiating vasoconstriction, sodium retention and diuresis. In local tissues, in contrast, the regulatory actions of the system appear to influence expression and synthesis of cellular proteins, the release of other autacoids and oxygen radical species, and even modulate the expression and function of other G-coupled transmembrane receptors. We refer to these local systems as having *paracrine*, *autocrine* and *intracrine* actions. Critical discoveries that expand our understanding of the role of the system in the regulation of cardiovascular function and cellular activity are documented as follows.

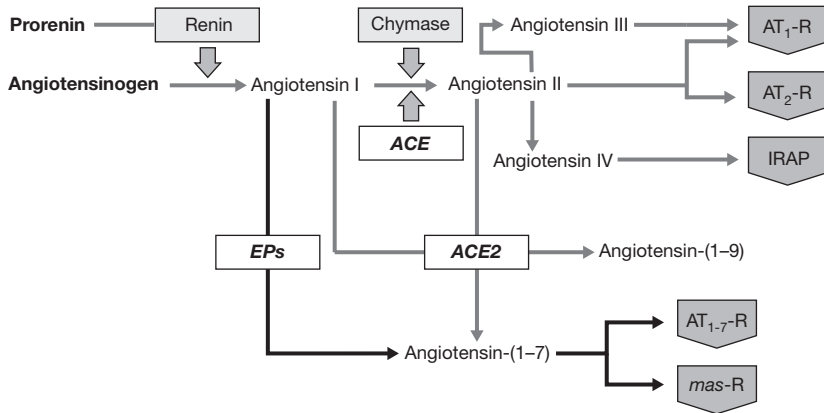


Fig. 1.1 Current schematic diagram of the biochemical pathways for the formation of angiotensin peptides and their binding to specific receptor subtypes. ACE, angiotensin-converting enzyme; Eps, endopeptidases (neutral endopeptidase 24.11; prolyl endopeptidase 21.26; metallo endopeptidase 24.15); ACE2, angiotensin-converting enzyme 2; AT₁-R and AT₂-R, angiotensin II receptor subtypes 1 and 2, respectively; AT₁₋₇-R, angiotensin-(1-7) subtype receptor; IARP, cyclic insulin aminopeptidase protein/AT₄ receptor.



Prorenin induces intracellular signalling in cardiomyocytes independently of angiotensin II

Saris JJ, 't Hoen PA, Garrelds IM, *et al.* *Hypertension* 2006; **48**: 564

BACKGROUND. The mechanism of excessive plasma concentrations of the renin precursor prorenin remains unresolved, but it has been suggested that they are indicative of tissue accumulation, leading to local renin activation and production of bioactive angiotensins. The discovery of a prorenin receptor has led to the suggestion that the excess prorenin may bind to the receptor, eliciting non-proteolytic activation and subsequent angiotensin production [1]. Prorenin has demonstrated angiotensin-independent effects through its actions on DNA synthesis, thrombin generation via release of plasminogen activator inhibitor (PAI-1), and gene expression, differentiation, and apoptosis through mitogen-activated protein kinase (MAPK) activation [1,2]. Additionally, prorenin serves as an insulin-like growth factor 2 (IGF2) receptor agonist, resulting in internalization and cleavage to renin [3]. The authors of this report examined the angiotensin-independent effects of prorenin in neonatal rat cardiomyocytes.

INTERPRETATION. Upon confirming the presence of the prorenin receptor within the cardiomyocytes, the authors demonstrated that a combination of prorenin- and angiotensinogen-augmented PAI-1 secretion, similar to the effect noted with Ang-II treatment. The increased PAI-1 was blocked by eprosartan, suggesting an AT₁-mediated effect, depending on Ang-II generation. In contrast, prorenin dose-dependently elevated

p38 MAPK phosphorylation. Non-glycosylated prorenin similarly increased p38 MAPK phosphorylation, as did the lower doses of prorenin, indicating the absence of a role for IGF2 receptors. A comprehensive transcript profile analysis of cardiomyocytes using gene microarrays revealed a significant overlap between Ang-II- and prorenin-induced genes. However, 28 genes were prorenin specific, as inhibition of renin activity and Ang-II receptor blockade did not alter the effects of prorenin. p38 MAPK activation by prorenin occurred independently of Ang-II, which was assessed by the dose-dependent phosphorylation of HSP27 by prorenin. This activity was blocked using the p38 MAPK inhibitor SB203580. The activation of the p38 MAPK pathway ultimately led to alterations in the actin filaments, a known function of MAPK activity.

Comment

Prorenin appears to contribute to intracellular signalling independent of Ang-II and IGF receptors. Although the present data suggest the actions of prorenin are via MAPK pathway activation, the mechanism responsible for this activity has yet to be deciphered. The authors propose that a key component may be the prorenin receptor, which can lead to cardiovascular damage separate from hypertension, particularly in rats overexpressing prorenin. A caveat to consider in these studies is that the data, although quite compelling, were obtained from cardiomyocytes isolated from neonates, which may not mimic the physiology of adult myocytes.



Cardiac mast cell-derived renin promotes local angiotensin formation, norepinephrine release and arrhythmias in ischemia/reperfusion

Mackins CJ, Kano S, Seyedi N, et al. *J Clin Invest* 2006; **116**: 1063–70

BACKGROUND. Many studies have documented the presence of a local RAS independent of the circulating system, within the heart, which may have intra- or paracrine functions in nature [4,5]. This locally acting system is believed to contribute to pathophysiological conditions within the heart, such as myocardial ischemia. Previously, Silver and colleagues [6] localized renin protein in mast cells and demonstrated that once the renin was released from the cells, an obvious extrarenal source, it cleaved angiotensin I (Ang-I) from angiotensinogen. Ang-I was then readily available for conversion to Ang-II, which stimulated the sodium/hydrogen ion exchangers on sympathetic nerve terminals via norepinephrine (NE) release. Following that report, the authors attempted to determine if the release of mast cell renin, which is known to occur through myocardial ischemia-induced degranulation, leads to cardiac dysfunction by sequential NE release due to local RAS activation.

INTERPRETATION. In order to confirm that cardiac mast cells release active renin upon degranulation, the coronary effluent from Langendorff-perfused guinea pig hearts was assayed for renin activity following treatment with 48/80, a degranulating compound. Ang-I formation increased significantly following degranulation – an observation that

was blocked by the renin inhibitor BILA2157 – suggesting that renin is responsible for the angiotensinogen conversion to Ang-I. Following these experiments, isolated cardiac synaptosomes were treated with angiotensinogen and the release of NE was assessed to determine if the mast cell renin release could lead to local RAS activation. As expected, increasing doses of angiotensinogen led to increased NE release. This effect was augmented by treatment with the degranulating agent 48/80, and, in contrast, attenuated by mast cell stabilization with lodoxamide. Challenge with losartan and PD123319 – AT₁ and AT₂ receptor antagonists, respectively – revealed that the effect was AT₁ mediated, as losartan was able to block the effects while PD123319 had no effect.

Langendorff-perfused normal guinea pig and mouse hearts, as well as mast-cell deficient mouse hearts, revealed enhanced renin and NE release during reperfusion following pre-ischemia in the normal cardiac tissue, but not in the mast cell-deficient mice. These increases were associated with ventricular arrhythmias and were attenuated following mast cell stabilization and/or renin blockade.

Comment

Cardiac mast cell renin is potentially a cardiac dysfunction therapeutic target to ameliorate the consequence of ischemia–refusion. Extrarenal renin, which is active upon its release from the mast cells, has clearly been shown to activate a local RAS within the heart, resulting in Ang-II generation and subsequent NE release. These actions were shown to be AT₁ dependent and were exacerbated following ischemia, which led to ventricular fibrillation and tachycardia. The authors suggest that mast cell stabilization, renin blockade and inhibition of AT₁ activity lessened the effects of the local actions of cardiac renin, but that these compounds were not completely sufficient. This suggests additional input from other sources, such as bradykinin, and these mechanisms require further examination.



Elevated blood pressure and heart rate in human renin receptor transgenic rats

Burcklé CA, Jan Danser AH, Müller DN, *et al.* *Hypertension* 2006; **47**: 552–6

BACKGROUND. Intense investigations pursuing the pertinent role of renin-specific receptors – the mannose-6-phosphate/IGF2 receptor and the renin/prorenin-specific receptor – propose that the functionality can be a mechanism either for clearance or for angiotensin-independent actions such as intracellular signalling and prorenin activation [1,3,7,8]. Using transgenic rats that overexpressed the human renin receptor gene in smooth muscle, this study examined the role of the renin receptor on local and systemic RAS activation in this model of delayed hypertension and tachycardia, in addition to elevated aldosterone secretion.

INTERPRETATION. The highest expression of the renin receptor gene was isolated to the aorta, bladder, uterus, and lung tissues, which have a high smooth muscle content. Incubation of aortic segments, whose vascular smooth muscle cells exhibited significant transgene mRNA expression with human prorenin, resulted in the uptake of prorenin into

the vascular wall. Phenotypically, hypertension and heart rate elevation occurred with an onset at around 6 months of age in the transgenic rats. The plasma renin concentration and activity, similar to renal function, were not altered by the presence of the transgene. Aldosterone was significantly elevated in the transgenic rats, but appeared to be adrenal in origin as Ang-I and plasma renin concentration and activity were essentially the same in control and transgenic animals.

Comment

Overexpression of the human renin receptor gene in smooth muscle led to later-onset hypertension and hyperaldosteronism, independent of systemic Ang-II formation. This potential intra-adrenal RAS activation is likely responsible for the cardiovascular phenotype. These data highlight therapeutic opportunities to reduce cardiovascular damage elicited by the hypertension and hypokalemia resulting from hyperaldosteronism, by way of renin or prorenin blockade through interference of the specific receptor.



Intracellular angiotensin II induces cell proliferation independent of AT₁ receptor

Baker KM, Kumar R. *Am J Physiol Cell Physiol* 2006; **291**: C995–C1001

BACKGROUND. Baker *et al.* [9] previously demonstrated that *in vitro* overexpression of intracellular Ang-II in cultured myocytes of mouse neonates leads to cellular hypertrophy. Concurrent *in vivo* experiments revealed significant cardiac hypertrophy in mice overexpressing intracellular Ang-II. Of particular interest in the previous study was that AT₁ blockade with losartan did not block the hypertrophic response. In order to decipher the role of the AT₁ receptor in Ang-II's paracrine actions, the present study utilized AT₁ and/or intracellular transfected Chinese hamster ovary (CHO) cells to examine cellular proliferation.

INTERPRETATION. As illustrated by receptor-binding experiments, the normal CHO cells lacked AT₁ receptors, while the transfected cells abundantly expressed the Ang-II receptor subtype. This binding was blocked by losartan, but not by the AT₂ antagonist PD123319, suggesting the presence of AT₁ receptors only. Ang-II concentrations in normal CHO cells and in the culture medium of both cell types were significantly lower than in the AT₁-transfected cells, leading to the conclusion that the Ang-II content was maintained intracellularly. In an attempt to characterize the effects of intra- vs. extracellular Ang-II on the proliferation of the transfected and normal CHO cells, the Ang-II expression vector was transiently transfected, resulting in increased growth in AT₁-transfected cells following exposure to extracellular Ang-II, but not in normal CHO cells. Both cell types exhibited enhanced growth following intracellular Ang-II exposure. A vast array of AT₁ antagonists blocked the extra- but not intracellular Ang-II effects. Additionally, cells containing Ang-II proliferate faster than those without intracellular Ang-II. Combining intra- and extracellular Ang-II further enhanced the proliferation of the AT₁-transfected cells, but not the normal CHO cells.

Comment

The data from this article suggest that the intracellular actions of Ang-II within cardiomyocytes occur independently of AT₁ receptor coupling. This observation complicates conventional angiotensin receptor blocker (ARB) therapy as treatment with ARBs, at least in this cellular model, does not interfere with intracellular Ang-II activity. Therefore, further examination of tissue-specific, or more precisely intracellular-specific, mechanisms of deleterious Ang-II actions is required in order to overcome pharmacological barriers that prevent the intracrine blockade of the system.



Evidence supporting a functional role for intracellular renin in the brain

Lavoie JL, Liu X, Bianco RA, et al. *Hypertension* 2006; **47**: 461–6

BACKGROUND. Following numerous reports that locally acting tissue RAS involving renin, angiotensinogen and Ang-II type 1 receptors seem to initiate pathological conditions through disturbances in cellular growth and function, the authors of this report sought to determine the role of renin localized within the brain in the maintenance of fluid homeostasis and regulation of blood pressure [10]. Previously, this group of investigators have demonstrated a form of renin transcript different from the conventional mRNA [11]. The altered form is believed to be constitutively active intracellularly as it lacks the signalling molecule on the propeptide. In these studies, this particular form was evaluated for its actions in double-transgenic mice expressing human angiotensinogen and intracellular renin or secreted renin.

INTERPRETATION. Mice expressing intracellular renin were bred and, following confirmation that no renin was being released into the cerebrospinal fluid, the mice were maintained in metabolic cages so that water intake and urine output could be monitored. Both transgenic mice expressing intracellular renin and those expressing secreted renin exhibited increased thirst compared with wild-type mice. This increase appeared to be greater in mice expressing secreted renin. Interestingly, urine output in the mice expressing intracellular renin did not increase above the levels measured in wild-type mice. However, urinary excretion significantly increased in the renin-secreting mice. The mean arterial pressure, as assessed by radiotelemetry, was elevated in both transgenic mice groups. Administration of losartan into the intracerebroventricular space decreased the blood pressure in both transgenic strains, but not in the non-transgenic littermates. Meanwhile, intravenous administration of losartan had no effect in all animals.

Comment

This report presents interesting functional data on the role of intracellular renin, derived from the newly discovered renin mRNA that lacks the signalling molecule of conventional renin transcripts. The augmentation of blood pressure in two variations of mice expressing human renin in the brain, either intra- or

extracellularly, was normalized only by intracerebroventricular injections of the AT₁ receptor antagonist losartan, and not by systemic exposure to the drug. Functionally, this supports the notion that the elevated pressures in this scenario result from local actions of Ang-II rather than a global circulating function. Although it seems quite obvious now that that intracellular renin, in its altered form, can cleave angiotensinogen to Ang-I to begin the cascade towards active peptide generation, the enigma of the location of Ang-II generation remains. In some reports, Ang-I conversion to Ang-II appears to occur intracellularly, but the data in this report do not confirm this because the Ang-II could be formed inside or outside the cell, then released to act upon the AT₁ receptors.



ACE2 activity is increased in monocyte-derived macrophages from prehypertensive subjects

Keider S, Strizevsky A, Raz A, et al. *Nephrol Dial Transplant* 2007; 22: 597–601

BACKGROUND. Emerging evidence shows that, in addition to angiotensin-converting enzyme (ACE), its homologue, ACE2, plays a critical role in Ang-II metabolism, generating counteracting angiotensin (1–7) [Ang-(1–7)] with potent vasodilator effects. Ang-(1–7) may also be generated indirectly by ACE2-mediated hydrolysis of angiotensin I (Ang-I) into Ang-(1–9) with its subsequent conversion to Ang-(1–7) by ACE. The purpose of this study was to examine ACE and ACE2 activities in human monocyte-derived macrophages (HMDMs) taken from normotensive, prehypertensive, and untreated hypertensive subjects in order to test the hypothesis that altered balance between these two enzymes is important in blood pressure control. The activities of ACE2 were determined by measuring leucine and phenylalanine released following hydrolysis of Ang-I and Ang-II, respectively. The activity of ACE was measured using a synthetic substrate.

INTERPRETATION. The analysis of the ACE activities in HMDMs in three groups of subjects revealed no change in ACE2-mediated hydrolysis of Ang-I or ACE activity among the study categories. However, ACE2-mediated Ang-II hydrolysis in prehypertensive people was significantly increased compared with both the normotensive and hypertensive groups. The authors concluded that, as no difference was found in ACE activity between blood pressure categories, the observed increased ACE2 activity would be protective in the early stage of hypertension development, shifting the angiotensin balance towards vasodilatory Ang-(1–7). Additionally, as macrophage infiltration and activation contribute importantly to development of target organ damage in hypertension, increased ACE2 activity in these cells may attenuate the progression of the disease.

Comment

The term ‘prehypertension’ was introduced in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, and replaces the old term of ‘high-normal blood pressure’ [12]. The

initiative was based on epidemiological data demonstrating that these patients are more likely to develop hypertension than the subject with optimal blood pressure. Moreover, risk factors for hypertension (age, obesity, diabetes) are more common in this stratum. Importantly, prehypertensive subjects who exhibit an exaggerated pressure response to exercise already show signs of cardiovascular remodelling and dysfunction [13]. A large multicentre study, in which prehypertensive subjects were medicated with candesartan for two consecutive years, supports this view, as the treatment was associated with a 33% decrease in the development of hypertension when compared with those subjects who received placebo [14]. Given these facts, Keidar's study, indicating that ACE2 activity is increased in monocyte-derived macrophages from prehypertensive subjects, provides an important new insight into the mechanisms underlying hypertensive disease. Despite its limitations, such as the limited number of subjects and the fact that only one type of cells were studied, the report is significant because macrophages are deemed to be one of the key players in the development of hypertensive heart disease. Thus, the increased ACE2 activity observed in HMDMs may represent a compensatory protective response put in place to favour cardiac Ang-II degradation to vasodilator and antiproliferative Ang-(1-7). Once that protection fails, hypertension and related cardiac remodelling and dysfunction develop.



Effects of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats

Jessup JA, Gallagher PE, Averill DB, *et al.* *Am J Physiol* 2006; **291**: H2166–H2172

BACKGROUND. The aim of this study was to investigate the effects of Ang-II blockade on the opposing arm of the RAS comprising vasodilatory Ang-(1-7) and its forming enzyme ACE2. The study was conducted on Lew.Tg(mRen-2) rats, an improved model of renin-dependent hypertension that was created by expression of the Ren-2 gene in Lewis rats. Angiotensin blockade was achieved by treating rats with either the ACE inhibitor, lisinopril, or the Ang-II receptor blocker, losartan, for 12 days. Changes in blood pressure as well as urinary output were recorded daily. Angiotensin concentrations were measured in arterial blood and urine samples. In addition, mRNA levels for ACE and ACE2 were determined in cardiac tissue and kidneys by reverse transcription polymerase chain reaction (RT-PCR) and related to enzyme activities.

INTERPRETATION. Normalization of blood pressure in this new congenic model of hypertension achieved by either lisinopril or losartan treatment was associated with significant increases in cardiac and renal ACE and ACE2 expression as well as protein activity of both serum ACE and tissue ACE2. While ACE inhibition reduced plasma Ang-II levels and increased plasma Ang-(1-7), the increases in plasma concentrations of Ang-II and Ang-(1-7) induced by losartan were, surprisingly, of borderline statistical significance.

In addition, in contrast to the normotensive animals, Ang-II blockade in hypertensive rats decreased urinary excretion of Ang-II and Ang-(1-7). The authors concluded that the paradoxical effects of Ang-II blockade on urinary peptides suggest that in Lew.Tg(mRen-2) rats there is a failure of compensatory ACE2/Ang-(1-7) vasodepressor mechanisms in buffering the Ang-II-dependent progression of hypertension.

Comment

This paper introduced a congenic model of renin-dependent hypertension. It was shown that the Lew.Tg(mRen-2) congenic hypertensive rat strain, developed through a backcross of hypertensive (mRen-2)²⁷ transgenic with normotensive Lewis rats, provides an excellent experimental model of gradually developing hypertensive heart and renal disease. In response to Ang-II blockade, normalization of the blood pressure was achieved and was associated with increased plasma Ang-(1-7) levels and increased cardiac and renal ACE2 expression and protein. This confirms previous findings of the important contribution of this vasodilator/antiproliferative peptide to the beneficial effects of Ang-II blockade [15,16]. As proposed by Ferrario *et al.* [17] (Fig. 1.2), ACE inhibition may not only reduce Ang-II formation but may also contribute to the accumulation of Ang-(1-7) by preventing degradation of Ang-(1-7) to the inactive Ang-(1-5). On the other hand, the increase in Ang-II due to prevention of the coupling of the peptide to the AT₁ receptor would provide substrate in excess for its ACE2-mediated conversion to Ang-(1-7). This concept reinforces our appreciation that ACE/ACE2 balance represents one of the critical determinants that may significantly shift the balance between two counteracting peptides, Ang-II and Ang-(1-7). The fact that, compared with the normotensive counterparts [18], the effects of the treatment on the ACE2/Ang-(1-7) axis were significantly attenuated in hypertensive animals

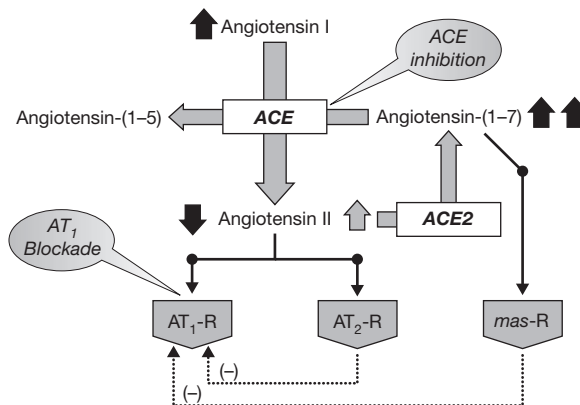


Fig. 1.2 Site of action of angiotensin-converting enzyme inhibitors and AT₁ receptor blockers with arrows pointing to directional changes in activity of angiotensin-forming enzymes and peptide concentrations. Source: Ferrario CM, Jessup JA. *Curr Hyperten Rev* 2007; **3**: 97-104.

gives support to the concept that prohypertrophic and profibrotic actions of Ang-II in hypertension occur on a background of reduced counterbalancing effects of ACE2/Ang-(1-7). Interestingly, in response to RAS blockade, compensatory ACE upregulation was observed in hypertensive but not normotensive animals. Therefore, the residual (after ACE inhibition) or excessive (after AT₁ receptor blockade) Ang-II formation may have inhibited ACE2 expression as it has been shown that Ang-II, via AT₁ receptors, negatively regulates ACE2 expression in the heart, kidney and astrocytes [18-20]. Elucidation of the difference in these regulatory mechanisms between normotensive and hypertensive subjects requires further investigation.



Primary role of angiotensin-converting enzyme 2 in cardiac production of angiotensin (1-7) in transgenic Ren-2 hypertensive rats

Trask A, Averill DB, Ganten D, et al. *Am J Physiol* 2007; **292**: H3019-H3024

BACKGROUND. The newly discovered homologue of angiotensin-converting enzyme (ACE), ACE2, represents a critical pathway for Ang-II metabolism into vasodilatory and antiproliferative Ang-(1-7). Heart expresses ACE2, and in this study the authors examined the extent to which this enzyme is involved in cardiac conversion of Ang-II into Ang-(1-7). Given the possibility that hypertrophic and profibrotic effects of Ang-II may be facilitated by a reduction in the counterbalancing action of Ang-(1-7) in hypertensive heart, the ACE2 pathway characterization was attempted in isolated hearts from both normotensive (Sprague-Dawley) and hypertensive (mRen-2)²⁷ transgenic animals utilizing a Langendorff preparation.

INTERPRETATION. During a 60-min recirculation period with 10nmol/l Ang-II, the presence of Ang-(1-7) was assessed in the cardiac effluent. Ang-(1-7) generation from Ang-II was similar in both normal and hypertensive heart. However, ACE2 inhibition significantly reduced Ang-(1-7) production in hypertensive but not in normotensive heart. These results confirm previous findings in humans that cardiac tissue has a capacity to generate Ang-(1-7) from Ang-II. Importantly, this study extended those findings by revealing a very significant dependence of Ang-(1-7) formation on ACE2 in the hypertensive heart, whereas ACE2 had no major role in Ang-(1-7) formation in the normal heart. The authors concluded that ACE2 may serve as a compensatory mechanism to preserve Ang-(1-7) levels in hypertension-induced cardiac hypertrophy, although it may not be sufficient to counteract the deleterious effects of Ang-II.

Comment

The importance of this article lies in the fact that the difference in Ang-II metabolic pathways between hypertensive and normotensive heart was demonstrated for the first time. ACE2 was predominantly responsible for Ang-II conversion to Ang-(1-7)

in hypertensive heart whereas some other, yet to be identified, enzyme(s) bear(s) that responsibility in the normal heart. It should be noted that a difference in ACE activity between hypertensive and normotensive hearts cannot be completely ruled out as an explanation of these findings because ACE rapidly degrades Ang-(1-7). However, this is less likely since Ang-(1-7) formation from Ang-II was similar between two strains. Therefore, determination of the enzymatic pathway that contributes to Ang-(1-7) formation from Ang-II in normal heart will provide new mechanistic insight into the role of RAS in cardiac growth and function and further our understanding of its role in diseased heart. New therapeutic strategies may then address ways to augment these Ang-(1-7)-forming activities and/or signalling through the Ang-(1-7) receptor. As ACE2 activity found in cardiac effluent in this study represents mostly the activity of enzyme related to coronary vascular bed, the tissue contribution of different enzymes in Ang-(1-7) generation from Ang-II should also be explored in further studies.



Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain RAS

Doobay MF, Talman LS, Obr TD, et al. *Am J Physiol* 2007; **292**: R373–R381

BACKGROUND. The brain RAS has a considerable role in pathogenesis of cardiovascular diseases such as hypertension. The primary aim of this study was to characterize the regional distribution and cellular expression of ACE2 in the central nervous system of mice. Whether its expression was regulated by the other components of the RAS was also examined using two different transgenic mouse models that exhibit alteration of the brain RAS components. Therefore, the immunohistochemical localization and gene expression of ACE2 were examined in normal and transgenic mice with either widespread expression of both human renin and angiotensinogen genes or brain-selective overexpression of the rat Ang-II type 1A receptor.

INTERPRETATION. Using cell type-specific antibodies, ACE2 was observed in the cytoplasm of neuronal cells bodies but not in glial cells. The results also showed that ACE2 staining was widely distributed throughout the brain, predominantly in the area involved in central regulation of cardiovascular function. Furthermore, compared with the control counterparts, the ACE2 level was decreased in nucleus tractus solitarius but increased in rostroventrolateral medulla in hypertensive transgenic mice harbouring human renin and angiotensinogen genes. These results parallel the previously observed functional response to Ang-(1-7) infusion in those baroreflex areas, providing additional evidence for ACE2 involvement in cardiovascular function. In both transgenic mice models, ACE2 was increased in the subformal area, a region of the brain critically involved in the regulation of blood pressure and volume homeostasis. Therefore, ACE2 seems to have a similar role as in periphery to counterbalance the excess in Ang-II in nuclei involved in cardiovascular and autonomic regulation.

Comment

ACE2 expression was originally identified in the heart, kidney, and testis. Later, its distribution was extended to respiratory and gastrointestinal tracts, skin, lymph nodes, and hematopoietic tissue. This study was the first to show widespread distribution of the Ang-II degradation enzyme throughout the brain. Altered ACE2 presence in brain regions relevant for cardiovascular and autonomic regulation in transgenic mice with overexpressed RAS components gives support to the concept that changes in ACE2/Ang-(1–7) axis relative to ACE/Ang-II facilitate enhanced sympathetic flow and reduce vagal outflow and development of hypertension.



Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin II-dependent glomerulosclerosis

Oudit GY, Herzenberg AM, Kassiri Z, et al. *Am J Pathol* 2006; **168**: 1808–20

BACKGROUND. Renal expression of ACE2, which is abundant in the normal kidney, is decreased in hypertensive rodent models, suggesting its potential role in hypertension-related kidney disease. To further investigate the role of ACE2 in renal disease the impact of targeting disruption of ACE2 on kidney structure and function was investigated in male (ACE2^{-y}) and female (ACE2⁻⁺) mutant mice. To test the hypothesis that deletion of the ACE2 gene would be associated with Ang-II-dependent glomerular injury, one group of male mutant (ACE2^{-y}) mice were treated with the Ang-II receptor antagonist irbesartan. Treatment was initiated at 9–10 weeks of age and maintained until 1 year of age. Kidneys were examined under light and electron microscopy in order to identify early structural and ultrastructural abnormalities (glomerular sclerosis, fibrosis). Blood pressure and urinary protein electrophoresis were measured to assess functional consequences of ACE2 deletion.

INTERPRETATION. Targeted disruption of ACE2 resulted in early fibrillar collagen deposition in the glomeruli that progressed towards glomerular sclerosis over the course of the experiment in male but not in female mutant mice. However, renal function was preserved in male mutant mice, and the glomerular injury that was not related to blood pressure or blood glucose level resulted from increased oxidative stress and activation of mitogen-activated protein kinase. AT₁ receptor antagonism prevented albuminuria and development of oxidative stress and resulting glomerulosclerosis, hyalinosis, capillary microaneurysm, and mesangial expansion in male mutant mice, suggesting the role of altered Ang-II processing due to lack of its ACE2-mediated metabolism. Previous work from the same group already demonstrated that the loss of ACE2 leads to increase in plasma and renal Ang-II levels [21]. Finally, the authors emphasized that ACE2 deletion in female mutant mice did not result in glomerular injury, giving support to the protective role of estrogen against Ang-II-induced glomerular injury.

Comment

In this paper Ang-II-dependent glomerulosclerosis was documented as a consequence of the loss of ACE2 and supported further a view that non-functional ACE2 favours increased renal Ang-II levels through reduced conversion to Ang-(1-7). Although Ang-(1-7) levels in the kidney tissue were not measured in this study, one would expect that ACE2 depletion would lead to reduced antihypertrophic and antiproliferative Ang-(1-7) potential. This study was an extension of the original report in which severe cardiac dysfunction was described in ACE2 knock-out mice of both genders [22]. Interestingly, targeted disruption of ACE2 leads to glomerulosclerosis in male but not female ACE2 mutant mice, suggesting a renal protective role of estrogen. Indeed, over the years, the gender disparity in incidence of adverse cardiovascular and renal events has been interpreted primarily in the milieu of estrogen-mediated prevention of heart and kidney disease [23]. Therefore, it would be of great interest to further investigate mechanisms responsible for female gender protection against the pathological effects of Ang-II in terms of structural alteration in kidneys and why they are not effective in heart under conditions of ACE2 depletion. Furthermore, glomerular injury in ACE2 mutant mice seems to develop independently of blood pressure or glucose level changes. Interestingly, the number of hypertensive patients with end-stage renal disease (ESRD) has increased over the past decades, despite the reported beneficial effects of antihypertensive therapy in preventing and reducing morbidity and mortality from stroke and coronary artery disease. Although cerebral and coronary circulation may be more sensitive to the reduction of blood pressure, data from this study suggest that it may be equally important to achieve the blood pressure goal as well as to correct altered local tissue Ang-II/Ang-(1-7) balance. Notably, a majority of the patients who progressed to ESRD were patients with hypertension and/or diabetes, pathological conditions already characterized by reduced ACE2 potential.



Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney

Crowley SD, Gurley SB, Herrera MJ, et al. *Proc Natl Acad Sci USA* 2006; **103**: 17985–90

BACKGROUND. Although much research has focused on elucidating the causative factors of hypertension, the etiology of the disease still remains largely unknown. Undoubtedly, the angiotensin AT₁ receptor is involved in the pathophysiology of hypertension, as blocking it can improve blood pressure, cardiac hypertrophy, and kidney function. In the current study, Crowley et al. wanted to separate the renal AT₁ receptor pool from the systemic AT₁ receptor pool in an attempt to elucidate a mechanism of hypertension. To do so, the investigators cross-transplanted kidneys from C57BL/6×129 wild-type mice and AT_{1A} receptor knock-out mice.

INTERPRETATION. The authors found that, by transplanting an AT_{1A} knock-out kidney into bilaterally nephrectomized wild-type mice (kidney KO), the systemic AT_{1A} receptors of the kidney KO mice were not able to induce hypertension or cardiac hypertrophy in response to a 4-week Ang-II infusion. Moreover, transplantation of a wild-type kidney into the bilaterally nephrectomized AT_{1A} knock-out mouse (systemic KO) resulted in a marked elevation in mean arterial pressure and cardiac hypertrophy in response to chronic Ang-II. These data provide evidence that the renal AT_{1A} receptor is required for the development of hypertension and target organ damage.

Comment

The data presented in this article strongly suggest the involvement of the *renal* AT_{1A} receptor in the pathogenesis of hypertension and cardiac hypertrophy. The finding that renal AT_{1A} receptors are necessary for the development of hypertension is not a surprising one, as the kidney is the long-term regulator of blood pressure. However, given that others [24–27] have shown that cardiac hypertrophy is mediated by cardiac AT_1 receptors *in vitro*, the finding that renal AT_{1A} receptors are necessary for the development of cardiac hypertrophy goes against current dogma. Indeed, it is well accepted that cardiac hypertrophy can be reversed by angiotensin receptor blockers (ARBs), which has been attributed to the blockade of the cardiac AT_1 receptors [28]. However, the findings of the current paper suggest that actions of ARBs administered for the treatment of cardiac hypertrophy and/or heart failure in both experimental models and patients may be due to blockade of the *renal* AT_1 receptors, which contradicts the idea that the reversal of cardiac hypertrophy is due to the blockade of cardiac AT_1 receptors. It is, therefore, imperative that the role of the cardiac AT_1 receptor is elucidated *in vivo* to determine whether the actions of ARBs are due to local cardiac blockade or if the actions are mediated solely through the renal AT_{1A} receptor pool. The current findings suggest that the renal AT_1 receptor may be at least partially mediating the etiology behind hypertension and cardiac hypertrophy, which undoubtedly requires further examination as it holds tremendous potential in uncovering the pathophysiology of hypertension.

Conclusion

Formidable progress continues to be achieved in dissecting the biochemical complexity of the RAS and understanding the multiplicity of actions by which the active products of the system regulate the cellular and endocrine mechanisms of cardiovascular regulation, in both normal and pathological states. New data on the actions of prorenin and the prorenin/renin receptor suggest a direct function of the enzyme in terms of cellular growth and development. These data might explain the occurrence of cardiovascular damage in rats overexpressing prorenin by mechanisms independent of Ang-II and blood pressure [29].

The ACE/Ang-II/AT₁ receptor arm of the RAS is negatively regulated by the ACE2/Ang-(1-7)-Mas receptor arm, eliciting signalling mechanisms that result in inhibition of protein synthesis and proliferation, inhibit collagen deposition, and counterbalance the effects of Ang-II in stimulating oxidative stress and inflammation. The role of AT₂ receptors in negatively regulating the agonistic effects of Ang-II appears to be exerted through their re-expression in disease states.

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