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Genetic models provide unique insight into angiotensin and bradykinin peptides in the extravascular compartment of the heart *in vivo*

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SUMMARY

1. There is continuing uncertainty about the tissue compartments where angiotensin and bradykinin peptide formation occurs. Mice with angiotensin converting enzyme (ACE) expression targeted to the cardiomyocyte membrane provide a unique experimental model to detect ACE substrates in the extravascular compartment of the heart *in vivo*.
2. Angiotensin I and II, bradykinin-(1–7), and bradykinin-(1–9) were measured in blood and cardiac ventricles of wild type (WT) mice; mice with a nonfunctional somatic ACE gene promoter (KO); mice homozygous (8/8) and heterozygous (1/8) for cardiomyocyte-targeted ACE expression and a nonfunctional somatic ACE gene promoter; and mice heterozygous for cardiomyocyte-targeted ACE expression and heterozygous for the WT ACE allele (WT/8).
3. Cardiac angiotensin II levels of 8/8, 1/8, WT/8, and WT mice were higher than KO levels. Cardiac angiotensin II levels in 8/8 and 1/8 mice were also higher than WT levels, but the levels in WT/8 mice were similar to WT levels. Cardiac bradykinin-(1–9) levels of WT, but not 8/8 mice, were lower than in KO mice, whereas bradykinin-(1–7) levels in 8/8 mice were lower than in KO mice.
4. We conclude that angiotensin I and bradykinin-(1–7) are present in the cardiac extravascular compartment of mice lacking vascular ACE, and extravascular ACE produces angiotensin II and metabolizes bradykinin-(1–7) in this compartment. These data suggest the vascular compartment is the main site of angiotensin I and bradykinin-(1–9) formation and metabolism, and vascular ACE may limit angiotensin I entry to the extravascular compartment of WT mice.

Keywords

Heart; angiotensin; bradykinin; angiotensin converting enzyme; genetic model

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Introduction

Angiotensin and bradykinin peptides play important roles in cardiac physiology and disease,[1–6] and the therapeutic effects of angiotensin converting enzyme (ACE) inhibitors and angiotensin type 1 receptor blockers (ARBs) are mediated in part by their modification of the levels and actions of these peptides in the heart.[1,2,4,5,7,8] Myocardial angiotensin and bradykinin peptide levels are higher than can be accounted for by the blood content of tissue,[9,10] and are consistent with peptide formation within the myocardium. There is, however, uncertainty about the location of angiotensin and bradykinin peptides in the myocardium and the contribution of the vascular and extravascular compartments to their formation and metabolism in this tissue (Figure 1).[6,11] Nephrectomy models established that kidney-derived renin is the main mechanism of formation of cardiac angiotensin peptides,[9,12] and studies of angiotensin production by the heart showed most cardiac angiotensin II (Ang II) is produced at tissue sites by conversion of *in situ*-produced, rather than blood-derived, angiotensin I (Ang I).[13] However, these studies did not identify the specific tissue compartments where Ang I is formed and converted to Ang II.

Study of peptides in the extravascular compartment of the heart presents special challenges because of the difficulties of access and sampling of this compartment *in vivo*. We recently reported the production of mice with cardiomyocyte-targeted ACE expression, in which the endogenous ACE gene was placed under the control of the α-myosin heavy chain promoter.[14] These mice offer the possibility to use cardiomyocyte-targeted ACE as a reporter for the presence of ACE substrates in the extravascular compartment of the heart *in vivo*. ACE converts Ang I to Ang II, bradykinin-(1–9) [BK-(1–9)] to bradykinin-(1–7) [BK-(1–7)], and BK-(1–7) to bradykinin-(1–5) [BK-(1–5)]. Ang II formation by cardiomyocyte-targeted ACE depends on Ang I having access to the extravascular compartment, due either to Ang I formation in the extravascular compartment or entry from the vascular compartment. Similarly, alteration in BK-(1–7) and BK-(1–9) levels by cardiomyocyte-targeted ACE indicates that these peptides have access to the extravascular compartment of the heart.

We present here the pooled data from 3 separate studies that comprised 5 genetic models of ACE gene expression in mouse heart. These were wild type (WT) mice, mice with a nonfunctional somatic ACE gene promoter (KO), mice homozygous (8/8) and heterozygous (1/8) for cardiomyocyte-targeted ACE expression and a nonfunctional somatic ACE gene promoter, and mice heterozygous for cardiomyocyte-targeted ACE expression and heterozygous for the wild type ACE allele (WT/8). Angiotensin peptide data from these mice were previously reported.[14–16] Bradykinin peptide data from WT, KO, and some 8/8 mice were also reported,[14,15] but bradykinin peptide data from 1/8, WT/8, and some of the 8/8 mice were not previously reported. Previous reports of these studies focused on the phenotype of the genetic models and the peptide data from 8/8, 1/8 and WT/8 mice were compared with WT levels. However, given that 8/8 and 1/8 mice with cardiomyocyte-targeted ACE expression had a nonfunctional somatic ACE gene promoter, the present study examined the compartmentalization of angiotensin and bradykinin peptides in the heart by comparing these genetic models with KO mice. Peptide data from the 8/8, 1/8 and WT/8 mice[14,16] were previously published separately from peptide data from the KO mice,[15] and this comparison was not reported.

Methods

The Bernstein laboratory used targeted homologous recombination to produce different genetic mouse models of targeted ACE expression, and the numbering system refers to the order in which these models were produced.[17,18] WT mice (n=40) were pooled from 3

separate studies.[14–16] KO mice (n=15) were from one study and included both ACE 1/1 mice (n=5) and ACE 4/4 mice (n=10).[15] ACE 1/1 mice are null for all ACE gene expression, producing neither somatic nor testis ACE.[19] ACE 4/4 mice have the somatic ACE gene promoter replaced by the kidney androgen-regulated protein (KAP) promoter. This modification was designed to produce androgen responsive ACE expression in the kidney.[17,20] However, the KAP promoter was essentially non-functional in ACE 4/4 mice and, in the absence of exogenous androgens, the levels of renal ACE were less than 1% of normal and no ACE was detected in organs other than the kidney. ACE 4/4 mice present with a phenotype nearly identical to that of ACE 1/1 mice, except the males have normal fertility because they express testis ACE. ACE 8/8 mice (n=15), from two studies,[14,16] were homozygous for the ACE gene under the control of the α-myosin heavy chain promoter, and the somatic ACE gene promoter was nonfunctional.[14] These mice express ACE on the cardiomyocyte membrane and have cardiac ACE levels approximately 100-fold higher than WT levels. ACE 8/8 mice also have ACE activity in lung and plasma at 43% and 56%, respectively, of WT levels, and have WT levels of ACE in testis. Immunocytochemical studies showed ACE expression on the cardiomyocyte membrane and absence of vascular ACE expression in the heart of 8/8 mice, whereas WT mice had both endothelial and adventitial ACE expression, but no detectable ACE expression by cardiomyocytes.[14] ACE 1/8 (n=11) and WT/8 (n=13) mice, from one study,[16] were compound heterozygotes prepared by mating ACE WT/1 and WT/8 mice.[16] ACE 1/8 mice were heterozygous for the ACE gene under the control of the α-myosin heavy chain promoter, and a nonfunctional somatic ACE gene promoter, whereas WT/8 mice were heterozygous for the ACE gene under the control of the α-myosin heavy chain promoter, and heterozygous for the ACE WT allele.[16] Cardiac ACE expression of 1/8 and WT/8 mice was approximately half that of 8/8 mice. A summary of the sites and levels of ACE expression in the different genetic models is shown in Table 1. ACE activity in the lung and plasma of 1/8 mice was 30% and 23%, respectively, of WT levels, whereas for WT/8 mice the levels were 79% and 75%, respectively, of WT levels.[16]

ACE activity was measured using the ACE-REA kit from American Laboratory Products Company, Ltd. (Alpco, Windham, NH) and was defined as that inhibited by captopril. Tissues were briefly homogenized at low speed in ACE homogenization buffer (50 mmol/L HEPES, pH 7.4, 150 mmol/L NaCl, 25 μmol/L $ZnCl_2$, and 1 mmol/L PMSF). These homogenates were centrifuged at 10,000 × g and the supernatant discarded. The pellets were then resuspended in ACE homogenization buffer containing 0.5% Triton X-100 and vigorously re-homogenized. The tissue homogenates were again centrifuged at 10,000 x g and the supernatants were used for ACE activity measurement. Protein concentration was measured using BCA Protein Assay reagent kit (Pierce, Rockford, IL). Ventricular ACE activities were 0.8±0.1 (mean±SEM) U/μg protein in WT mice in our initial study,[14] and were 4.1±0.7 U/μg protein in WT mice in a subsequent study.[16] Ventricular ACE activities were 104.5±4.5 U/μg protein in 8/8 mice, 59.5±0.8 U/μg protein in 1/8 mice, 61.5±0.4 U/μg protein in WT/8 mice, and were undetectable in KO mice.[14,16,19]

The details of these experimental models and the measurement of Ang I, Ang II, BK-(1–7), and BK-(1–9) in blood and cardiac ventricles have been described previously.[14–16] Mice were anaesthetized with a mixture of ketamine (125 mg/kg) and xylazine (12.5 mg/kg) administered by intra-peritoneal injection. Blood was collected from the inferior vena cava directly into a syringe containing 5 mL 4 mol/L guanidine thiocyanate (GTC) using a 25-gauge needle. The heart (both cardiac ventricles, without atria) was then rapidly and immediately rinsed briefly in cold isotonic saline, weighed, and homogenized in 5 mL GTC. The GTC blood and tissue homogenates were then frozen at −80°C and shipped in dry ice to St. Vincent's Institute of Medical Research where peptide measurements were performed using high performance liquid chromatography-based radioimmunoassays. All 4 peptides

were measured in the same high performance liquid chromatography run from each blood and tissue extract. Ang I and Ang II were measured with the same *N*-terminal-directed radioimmunoassay, and BK-(1–7) and BK-(1–9) were measured with the same *N*-terminal-directed radioimmunoassay. We calculated the Ang II/Ang I and BK-(1–7)/BK-(1–9) ratios because these ratios provide an index of the rate of conversion of Ang I to Ang II and BK-(1–9) to BK-(1–7), respectively, in blood and heart.

ACE 1/1, 4/4, and 8/8 mice were created by using targeted homologous recombination in R1 embryonic stem cells (derived from a 129/SVX129SvJ F1 embryo) that were implanted in blastocysts from C57BL/6 mice.[14,17,19] ACE 1/1 mice were back-crossed to the C57BL/6 strain for 7 generations and therefore had greater than 99% C57BL/6 background, except for the ACE locus that remained 129. ACE 4/4, 8/8, 1/8, WT/8, and WT mice were of mixed background of 129 and C57BL/6. Mice were 3–7 months of age. All mice were bred from appropriate heterozygous breeding stock. Both male and female mice were studied, and age and sex-matched littermate controls were used in all studies.

This is an analysis of pooled data from three separate studies.[14–16] The same investigators working in the same laboratories and using the same methodologies performed all studies. For each of the separate studies pooled for this report, tissue collection was spread over several months because of the time required for breeding the different genetic models. Two of these studies included 15 WT mice each,[15,16] and one study included 10 WT mice.[14] To control for between-experiment variation in peptide levels, we expressed the peptide levels from each study as the ratio to the level in WT mice for that experiment. Peptide data were logarithmically transformed when necessary to obtain similar variances among groups. Comparisons between genetic models were by analysis of variance and Fisher's Protected Least Significant Difference test. The probability value for significance was defined as $P<0.05$.

Results

Angiotensin and bradykinin peptides in blood

The mean levels (±SD) of angiotensin and bradykinin peptides in blood of WT mice from the three studies are shown in Table 2. The blood levels of angiotensin and bradykinin peptides in the 5 groups of mice are shown as the ratios to the levels in WT mice in Figure 2. In comparison with KO mice, ACE expression in 8/8, 1/8, WT/8, and WT mice increased blood Ang II levels. Blood Ang I levels in 8/8, WT/8, and WT mice, but not 1/8 mice, were less than in KO mice, and the blood Ang II/Ang I ratios of 8/8, 1/8, WT/8, and WT mice were higher than in KO mice, indicative of the conversion of Ang I to Ang II by vascular, plasma, and lung ACE in WT and WT/8 mice, and by the low level of plasma and lung ACE in 8/8 and 1/8 mice. The Ang II/Ang I ratios of 8/8 and 1/8 mice were less than in WT mice, consistent with the absence of vascular ACE in 8/8 and 1/8 mice.

In comparison with KO mice, ACE expression reduced blood BK-(1–7) and BK-(1–9) levels of 8/8, 1/8, WT/8, and WT mice, indicative of the role of ACE in the metabolism of both peptides. There were no differences in BK-(1–7) levels between 8/8, 1/8, WT/8, and WT mice, whereas the BK-(1–9) level of 1/8 mice was higher than in 8/8, WT/8 and WT mice. The blood BK-(1–7)/BK-(1–9) ratios of 8/8 and WT/8 mice were not different from KO mice, whereas the BK-(1–7)/BK-(1–9) ratio of WT mice was higher than in KO mice, and the BK-(1–7)/BK-(1–9) ratio of 1/8 mice was less than in KO mice. The BK-(1–7)/BK-(1–9) ratios of 8/8 and 1/8 mice were less than in WT mice, consistent with the absence of vascular ACE in 8/8 and 1/8 mice.

Angiotensin and bradykinin peptides in the heart

The mean levels (±SD) of angiotensin and bradykinin peptides in cardiac ventricles of WT mice from the three studies are shown in Table 2. The cardiac levels of angiotensin and bradykinin peptides in the 5 groups of mice are shown as the ratios to the levels in WT mice in Figure 3. In comparison with KO mice, cardiomyocyte-targeted ACE expression caused large increases in cardiac Ang II levels, without change in Ang I levels, and increased Ang II/Ang I ratios, indicating increased Ang I conversion to Ang II in the cardiac extravascular compartment of 8/8 and 1/8 mice. WT mice also had higher cardiac Ang II levels and higher Ang II/Ang I ratios than KO mice, consistent with ACE expression in cardiac vasculature of these mice. Cardiac Ang II levels and Ang II/Ang I ratios of 8/8 and 1/8 mice were higher than in WT mice. By contrast, when cardiomyocyte-targeted ACE expression was combined with vascular ACE expression in WT/8 mice, cardiac Ang II level and Ang II/Ang I ratio were not different from WT mice, and were less than in 1/8 and 8/8 mice.

In comparison with KO mice, cardiomyocyte-targeted ACE expression reduced cardiac BK-(1–7), but not BK-(1–9), levels in 8/8 mice. Cardiac BK-(1–7) level of WT/8 mice was also lower than in KO mice, and the BK-(1–7)/BK-(1–9) ratios of all three models of cardiomyocyte-targeted ACE expression (8/8, 1/8, WT/8) were less than in KO mice, indicative of increased BK-(1–7) metabolism by cardiomyocyte-targeted ACE. Cardiomyocyte-targeted ACE expression in 8/8 and 1/8 mice did not alter cardiac BK-(1–9) levels, in comparison with KO mice, suggesting that BK-(1–9) did not have access to the high ACE levels in the extravascular compartment of 8/8 and 1/8 mice. By contrast, cardiac BK-(1–9) levels in WT and WT/8 mice were lower than in KO mice, indicating BK-(1–9) metabolism by vascular ACE.

Discussion

This study demonstrated for the first time how genetic models might provide information about compartmentalization of peptides *in vivo*. Cardiomyocyte-targeted ACE expression increased cardiac Ang II levels and reduced cardiac BK-(1–7) levels in mice lacking vascular ACE, thereby demonstrating that Ang I and BK-(1–7) were accessible to cardiomyocytes and therefore present in the extravascular compartment of the heart. Although plasma ACE levels of 8/8 mice were 56% of WT levels,[14] any contribution of plasma or vascular ACE to cardiac Ang II levels of 8/8 mice was likely to be small because cardiac Ang II levels were 20-fold higher than blood Ang II levels, and immunocytochemistry showed ACE was absent from the cardiac vasculature of 8/8 mice.[14]

In contrast to 8/8 and 1/8 mice, cardiac Ang II levels of WT/8 mice were not different from those of WT mice, despite the similar cardiomyocyte-targeted ACE expression in 1/8 and WT/8 mice.[16] This finding for WT/8 mice was in agreement with the findings of Tian et al.,[21] who reported that transgenic rats with 50-fold elevation of cardiac ACE activity due to targeted expression of the human ACE gene to ventricular cardiomyocytes of rats with normal vascular ACE expression had cardiac Ang II levels that were not different from the levels in control rats. Our data for WT/8 mice suggest vascular ACE, at a heterozygous level, was able to influence Ang II formation in the cardiac extravascular compartment, possibly by limiting the transfer of Ang I from the vascular to the extravascular compartment. Vascular ACE expression at the homozygous level of WT mice may be even more effective in restricting Ang I transfer to the extravascular compartment. These findings therefore suggest that the source of Ang I for Ang II formation by cardiomyocyte-targeted ACE in the extravascular compartment of 8/8 and 1/8 mice was likely to be the vascular compartment, rather than Ang I production in the extravascular compartment. This interpretation is supported by the lack of effect of cardiomyocyte-targeted ACE on cardiac

Ang I levels. Note that the vascular compartment we refer to includes both the intravascular space and the vessel wall (Figure 1).

We did not measure plasma renin and angiotensinogen levels in these mice. We previously showed that ACE KO mice had increased renin levels and reduced angiotensinogen levels in plasma,[22] and it is likely that 8/8 and 1/8 mice had renin and angiotensinogen levels similar to KO mice because they lacked vascular ACE and had reduced plasma ACE levels. Moreover, we previously showed WT/1 (ACE +/−) mice had similar plasma renin and angiotensinogen levels to WT mice,[22] and it is likely that WT/8 mice had similar renin and angiotensinogen levels to WT mice because they had vascular ACE. Blood Ang I and BK-(1–9) levels of 1/8 mice were higher than in 8/8 mice, and this was probably due to the lower plasma ACE levels, together with the absence of vascular ACE expression, in these mice. Plasma ACE levels were 23% of WT levels in 1/8 mice, in comparison with plasma ACE levels of 56% of WT levels in 8/8 mice (Table 1).

One consideration in the interpretation of the cardiac angiotensin peptide levels in these mice is whether the difference in cardiac Ang II levels between 1/8 and WT/8 mice was due to higher renin levels and higher blood Ang I levels in 1/8 mice. There are several arguments against this possibility. Firstly, marked increases in plasma renin levels are accompanied by marked decreases in plasma angiotensinogen levels, thereby attenuating any effect of increased renin levels on Ang I levels in blood.[22] Secondly, cardiac Ang II levels of 1/8 and WT/8 mice were at least 8-fold higher than blood Ang II levels, indicating that blood angiotensin peptide levels made little if any contribution to cardiac angiotensin peptide levels. Thirdly, despite marked cardiomyocyte ACE expression, the cardiac Ang II/Ang I ratio of WT/8 mice was no different from that of WT mice, and less than that of 1/8 mice. Higher plasma renin and blood Ang I levels of 1/8 mice cannot explain the difference in cardiac Ang II/Ang I ratio between 1/8 and WT/8 mice.

Previous attempts to measure angiotensin peptides in the interstitial compartment of the heart using microdialysis produced widely divergent results.[23,24] Dell'Italia et al.[23] reported Ang II levels of 6,333 fmol/mL in cardiac microdialysate from dog heart, whereas Schuijt et al.[24] reported Ang II levels below the limit of detection (<30 fmol/mL) in microdialysate from pig heart. Another approach to measurement of angiotensin peptides in the interstitial compartment was to collect interstitial transudate from the isolated perfused rat heart,[25] but there is uncertainty about the extent of angiotensin peptide formation during collection of transudate from this *in vitro* model. The genetic models used in the present study had the advantage that they were *in vivo* models and were relatively free of artefacts that may be associated with alternative experimental approaches to the study of the compartmentalization of peptides.

Our study provides important new insight into the contribution of the extravascular compartment to Ang II formation in the heart. Our data clearly show Ang II formation in the extravascular compartment of mice with cardiomyocyte-targeted ACE expression and absent vascular ACE expression due to a nonfunctional somatic ACE gene promoter. There would appear, however, to be several impediments to Ang II formation in the extravascular compartment of the WT cardiac ventricle. One impediment is the relative lack of ACE in the extravascular compartment of WT mice. Immunocytochemical studies showed immunostaining for ACE in the vascular endothelium and adventitia of WT mice, but no ACE expression on cardiomyocytes.[14] Studies in humans also showed an absence of immunostaining for ACE on cardiomyocytes of normal left ventricle, although there was strong staining of endothelial cells and the endocardium.[26] A second impediment to Ang II formation in the extravascular compartment is the minor contribution of non-ACE enzymes to Ang II formation, as was evident for KO mice. A third impediment is the supply of Ang I

to the extravascular compartment. Cardiomyocyte-targeted ACE expression in 8/8 mice, at 100-times total cardiac ACE expression in WT mice, produced only a 2- to 4-fold increase in cardiac Ang II levels above WT levels,[14,16] indicating that Ang I supply to the extravascular compartment was rate-limiting. Ang I supply to the extravascular compartment may be even less when ACE is expressed by the vasculature, as discussed above. These impediments to Ang II formation within the extravascular compartment of the normal cardiac ventricle suggest Ang II within this compartment may be derived predominantly from the vascular compartment. The same arguments imply that the vascular compartment is the main site of Ang I formation and conversion to Ang II in the heart, as summarized in Figure 4.

In contrast to the relative lack of extravascular ACE in normal cardiac ventricle,[14,26] extravascular ACE expression is increased in disease states such as cardiac hypertrophy, myocardial infarction, and heart failure, in animals and humans.[6,26–29] A corollary of our proposal that vascular ACE may limit Ang I entry to the extravascular compartment of the heart is that ACE inhibitor therapy may promote Ang I entry to the extravascular compartment where Ang I may be converted to Ang II by the increased extravascular ACE expression that occurs in cardiac hypertrophy, myocardial infarction, and heart failure.[6,26–29] These data therefore provide a rationale for the use of ACE inhibitor doses sufficient to inhibit extravascular ACE, or their combination with ARB therapy, in the treatment of these conditions.

The impact of cardiomyocyte-targeted ACE expression on cardiac BK-(1–7) and BK-(1–9) levels was less than its effect on cardiac Ang II levels, probably because peptidases other than ACE contribute to bradykinin peptide metabolism.[2,30,31] ACE contributes to BK-(1–9) conversion to BK-(1–7), and to BK-(1–7) degradation. The lower cardiac BK-(1–7) level in 8/8 mice and lower BK-(1–7)/BK-(1–9) ratio in 8/8 and 1/8 mice than in KO mice were consistent with cardiomyocyte-targeted ACE acting on BK-(1–7) in the extravascular compartment of the heart. In addition, cardiac BK-(1–7) levels in 8/8 and WT/8 mice, and BK-(1–7)/BK-(1–9) ratios in 8/8, 1/8, and WT/8 mice, were less than in WT mice, consistent with more extensive BK-(1–7) degradation by cardiomyocyte-targeted ACE than by vascular ACE. The reduction of cardiac BK-(1–9) levels below the levels in KO mice by vascular ACE expression in WT/8 and WT mice, but not by the 50- to 100-fold higher levels of cardiomyocyte-targeted ACE expression in 8/8 and 1/8 mice, suggests cardiac BK-(1–9) was predominantly located in the vascular compartment of the heart, and consequently suggests that the vascular compartment was the major site of BK-(1–9) and BK-(1–7) formation, and the source of BK-(1–7) in the extravascular compartment, as summarized in Figure 4.

Although providing new information about the compartmentalization of angiotensin and bradykinin peptides in the heart, the present methodology had limitations. We were unable to quantify the concentrations of angiotensin and bradykinin peptides in interstitial fluid. In addition, this methodology did not provide information about the flux of peptides through the different pathways of their formation and metabolism. The amount of peptide in a compartment at any time point is determined by the rate of its production, and also by the rate of its removal by peptidase activity and by exit from the tissue via the circulation. Alternative methodologies are required to determine the rate of peptide production and peptide half-life in different tissue compartments.

In conclusion, these genetic models provided a unique insight into angiotensin and bradykinin peptides in the extravascular compartment of the heart *in vivo*. They demonstrated that Ang I and BK-(1–7) were present in the extravascular compartment of the heart of mice lacking vascular ACE, where extravascular ACE expression produced Ang II

and metabolised BK-(1–7). These studies provided evidence that the vascular compartment is the main site of Ang I formation and conversion to Ang II in the normal heart, and that vascular ACE may limit Ang I entry to the extravascular compartment. These studies also provided evidence that the vascular compartment is the main site of BK-(1–9) formation and conversion to BK-(1–7) in the heart. Improved understanding of the differential regulation of angiotensin and bradykinin peptides in different compartments of the heart may lead to better targeting of therapies and improved patient outcomes.

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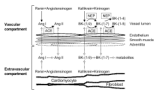

Figure 1.
Diagrammatic representation of the potential pathways of formation and metabolism of angiotensin and bradykinin peptides in the vascular and extravascular compartments of the heart. Solid arrows indicate the formation of angiotensin I (Ang I) and angiotensin II (Ang II) in the vascular compartment by the action of plasma renin on plasma angiotensinogen, and the conversion of Ang I to Ang II by endothelial angiotensin converting enzyme (ACE). Solid arrows also indicate the formation of bradykinin peptides by the action of plasma kallikrein on plasma kininogen, with subsequent conversion of bradykinin-(1–9) [BK-(1–9)] to bradykinin-(1–7) [BK-(1–7)], and of BK-(1–7) to BK-(1–5) by endothelial ACE. In addition, BK-(1–9) is converted BK-(1–7), and BK-(1–7) is converted to BK-(1–4) by endothelial neutral endopeptidase (NEP). Dashed arrows indicate possible pathways of formation and metabolism of angiotensin and bradykinin peptides in the extravascular compartment, and the possible transfer of peptides between the vascular and extravascular compartments.

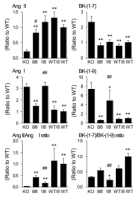

Figure 2.

Angiotensin II (Ang II), angiotensin I (Ang I), Ang II/Ang I ratio, bradykinin-(1–7) [BK-(1–7)], bradykinin-(1–9) [BK-(1–9)], and BK-(1–7)/BK-(1–9) ratio in blood of mice with a nonfunctional somatic angiotensin converting enzyme (ACE) gene promoter (KO), mice homozygous (8/8) and heterozygous (1/8) for cardiomyocyte-targeted ACE expression and a nonfunctional somatic ACE gene promoter, mice heterozygous for cardiomyocyte-targeted ACE expression and heterozygous for the wild type ACE allele (WT/8), and wild type mice (WT). Data expressed as ratio to mean WT value for each study, and shown as means ± SEM, n=15 for KO, n=15 for 8/8, n=11 for 1/8, n=13 for WT/8, and n=40 for WT mice. **P*<0.05, ***P*<0.01 vs KO mice; †*P*<0.05, ††*P*<0.01 vs WT mice; #*P*<0.05, ##*P*<0.01 vs WT/8 mice. In addition, there were significant differences between 8/8 and 1/8 mice for Ang I levels (P<0.01), Ang II/Ang I ratio (P<0.05), BK-(1–9) levels (P<0.01), and BK-(1–7)/BK-(1–9) ratio in blood (P<0.01).

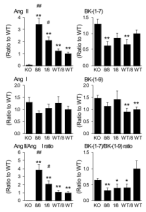

Figure 3.

Angiotensin II (Ang II), angiotensin I (Ang I), Ang II/Ang I ratio, bradykinin-(1–7) [BK-(1–7)], bradykinin-(1–9) [BK-(1–9)], and BK-(1–7)/BK-(1–9) ratio in cardiac ventricles of mice with a nonfunctional somatic angiotensin converting enzyme (ACE) gene promoter (KO), mice homozygous (8/8) and heterozygous (1/8) for cardiomyocyte-targeted ACE expression and a nonfunctional somatic ACE gene promoter, mice heterozygous for cardiomyocyte-targeted ACE expression and heterozygous for the wild type ACE allele (WT/8), and wild type mice (WT). Data expressed as ratio to mean WT value for each study, and shown as means ± SEM, n=15 for KO, n=15 for 8/8, n=11 for 1/8, n=13 for WT/8, and n=40 for WT mice. **P*<0.05, ***P*<0.01 vs KO mice; †*P*<0.05, ††*P*<0.01 vs WT mice; #*P*<0.05, ##*P*<0.01 vs WT/8 mice.

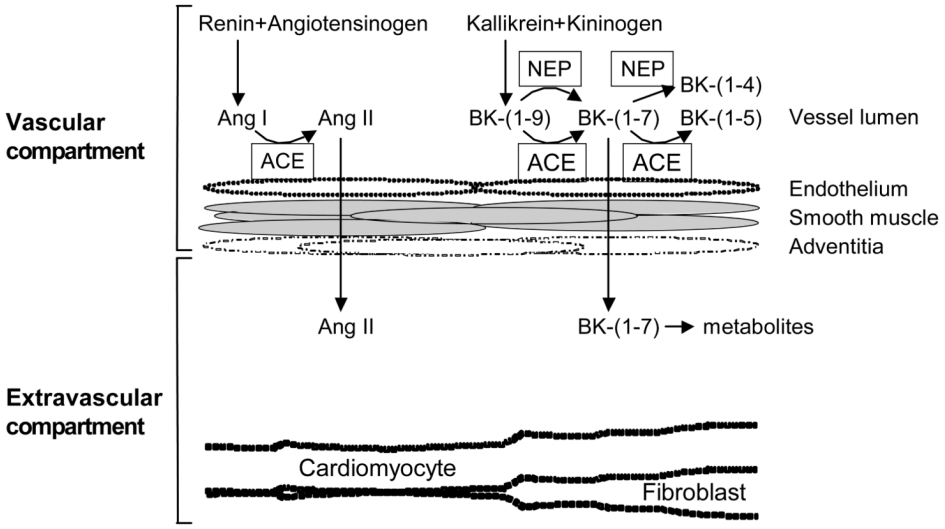

Figure 4.

Diagrammatic representation of the pathways of formation and metabolism of angiotensin and bradykinin peptides in the vascular and extravascular compartments of the heart, based on the interpretation of the cardiac peptide levels in the different genetic models. Arrows indicate the formation of angiotensin I (Ang I) and angiotensin II (Ang II) in the vascular compartment by the action of plasma renin on plasma angiotensinogen, and the conversion of Ang I to Ang II by endothelial angiotensin converting enzyme (ACE). Arrows also indicate the formation of bradykinin peptides by the action of plasma kallikrein on plasma kininogen, with subsequent conversion of bradykinin-(1–9) [BK-(1–9)] to bradykinin-(1–7) [BK-(1–7)], and of BK-(1–7) to bradykinin-(1–5) [BK-(1–5)] by endothelial ACE. In addition, BK-(1–9) is converted BK-(1–7), and BK-(1–7) is converted to BK-(1–4) by endothelial neutral endopeptidase (NEP). The vascular compartment is the main site of formation of Ang I, Ang II, BK-(1–9), and BK-(1–7). The vascular compartment is also the source of Ang II and BK-(1–7) in the extravascular compartment.

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Table 1

Summary of angiotensin converting enzyme expression by cardiac vessels and cardiomyocytes in the different genetic models.

Tissue site	Genetic model				
	KO	**8/8**	**1/8**	**WT/8**	**WT**
	n=15	**n=15**	**n=11**	**n=13**	**n=40**
Vascular ACE	-	-	-	0.5+	1+
Cardiomyocyte ACE	-	100+	50+	50+	-
ACE activity (% of WT level)					
Plasma	-	56%	23%	75%	100%
Lung	-	43%	30%	79%	100%

The level of angiotensin converting enzyme (ACE) expression by vessels and cardiomyocytes is shown in relation to a level of vascular ACE expression in WT mice of 1+. ACE was undetectable by immunocytochemistry in vessels of 8/8 and 1/8 mice, and in cardiomyocytes of WT mice. Data from previously published studies.[14-16]

Table 2

Mean peptide levels in blood and cardiac ventricles of wild type mice.

Peptide	Tissue	
	Blood	Cardiac ventricle
Ang II (fmol/mL or fmol/g)	10.1 ± 6.6	77.6 ± 62.8
Ang I (fmol/mL or fmol/g)	12.1 ± 12.3	5.2 ± 5.3
Ang II/Ang I ratio (mol/mol)	1.8 ± 3.6	22.8 ± 23.6
BK-(1–7) (fmol/mL or fmol/g)	11.4 ± 9.6	12.3 ± 7.8
BK-(1–9) (fmol/mL or fmol/g)	3.7 ± 10.7	6.1 ± 4.2
BK-(1–7)/BK-(1–9) ratio (mol/mol)	7.2 ± 6.5	3.5 ± 5.4

Data shown as means ± SD for the pooled data from three separate studies,[14–16] n = 40.