Effect of hypertension on angiotensin-(1–7) levels in rats with different angiotensin-I converting enzyme polymorphism

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Abstract

To determine circulating angiotensin-(1–7) [Ang-(1,7)] levels in rats with different angiotensin converting enzyme (ACE) genotypes and to evaluate the effect of hypertension on levels of this heptapeptide, plasma levels of angiotensin II (Ang II) and Ang-(1–7) were determined by HPLC and radioimmunoassay in (a) normotensive F_0 and F_2 homozygous Brown Norway (BN; with high *ACE*) or Lewis (with low ACE) rats and (b) in hypertensive F_2 homozygous male rats (Goldblatt model). Genotypes were characterized by PCR and plasma ACE activity measured by fluorimetry. Plasma ACE activity was 2-fold higher ($p<0.05$) in homozygous BN compared to homozygous Lewis groups. In the Goldblatt groups, a similar degree of hypertension and left ventricular hypertrophy was observed in rats with both genotypes. Plasma Ang II levels were between 300–400% higher ($p<0.05$) in the BN than in the Lewis rats, without increment in the hypertensive animals. Plasma Ang-(1–7) levels were 75–87% lower in the BN rats ($p<0.05$) and they were significantly higher ($p<0.05$) in the hypertensive rats from both genotypes. Plasma levels of Ang II and Ang-(1–7) levels were inversely correlated in the normotensive rats ($r=-0.64$; $p<0.001$), but not in the hypertensive animals. We conclude that there is an inverse relationship between circulating levels of Ang II and Ang-(1–7) in rats determined by the ACE gene polymorphism. This inverse relation is due to genetically determined higher ACE activity. Besides, plasma levels of Ang-(1–7) increase in renovascular hypertension.

Keywords: Angiotensin; Angiotensin-(1–7); Angiotensin converting enzyme polymorphism; Hypertension

Introduction

Angiotensin-(1–7) or Ang-(1–7) is formed from angiotensin I and II through the effect of tissue neutral endopeptidase (NEP) (Santos et al., 2000). Once Ang-(1–7) is formed it is rapidly hydrolyzed, mainly by angiotensin-I converting enzyme (ACE) (Chappell et al., 1998). Therefore, ACE inhibition should induce high levels of Ang-(1–7) as well as low levels of Ang II (Galvez et al., 2001). Ang-(1–7) increases the pharmacological effects of ACE inhibitors and those of angiotensin II type 1 receptor blockers (Santos et al., 2000). Recently, a novel angiotensin-converting enzyme (ACE)-related carboxypeptidase, ACE2, has been identified. In the human heart ACE2 degrades Ang II into Ang-(1–7) with high affinity. In this way, ACE2 is another peptidase that produces Ang-(1–7). Upregulation of ACE2 mRNA has been observed in rats after myocardial infarction by blockade of angiotensin II receptors that may be due to direct blockade of the AT_{1a} receptors or a modulatory effect of increased Ang-(1–7) (Ishiyama et al., 2004).

The effects of Ang-(1–7) are opposed to those of Ang II because Ang-(1–7) increases prostaglandin synthesis, potentiates the effects of bradykinins, produces nitric oxide and stimulates vasopressin liberation (Santos et al., 2000; Heitsch et al., 2001). Ang-(1–7) is an endogenous ligand for the G

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protein-coupled receptor Mas (a protooncogene). It has been observed that aortas from Mas-deficient mice do not relax in response to Ang-(1–7) (Santos et al., 2003). Thus, Mas has been identified as a functional receptor for Ang-(1–7) (Santos et al., 2003). The first evidence of an interaction between Ang-(1–7) with Ang II was made in 1989 describing that at the vascular level, Sar-Ang-(1–7), an Ang-(1–7) analog, inhibited the contractile effect of Ang II in the aorta from rabbits (Bovy et al., 1989). Other studies confirmed the antagonizing effect of Ang-(1–7) on the vascular effects of Ang II (Mahon et al., 1994; Roks et al., 1999; Ueda et al., 2000). Recent observations have been made on this antagonism between Ang-(1–7) and Ang II in human vessels (Ueda et al., 2000). Some evidences point to a direct interaction between Ang-(1–7) and the angiotensin II type 1 (AT_1) receptor (Mahon et al., 1994; Roks et al., 1999). Besides, high levels of Ang-(1–7) may produce similar effects as Ang II (Benter et al., 1995; Abbas et al., 1997). These actions could be explained by the low affinity of Ang-(1–7) to the AT_1 receptors (Rowe et al., 1995). It has been proposed recently in mesangial cells that Ang-(1–7) can bind to its specific receptors or to unknown AT_1 receptors (Chansel et al., 2001) and interfere with the calcium entry to the smooth muscle cell.

The presence of an ACE gene polymorphism in humans has been postulated from segregation analysis of plasma ACE levels in several families (Cambien et al., 1994). These variations have been correlated with different plasma and cellular ACE levels, probably modulating gene transcription. The insertion/deletion ACE gene polymorphism, characterized by the presence (insertion, I) or absence (deletion, D) of a fragment of 287 bp has been identified in the intron 16 of this gene (Soubrier et al., 1988). The ACE I/D polymorphism is associated to plasma and cellular ACE levels. The presence of the D allele is associated with higher ACE levels and vice versa (Jalil et al., 1999; Rigat et al., 1990; Tiret et al., 1992). In the rat, a similar ACE polymorphism has been described in the BP/SP-1 locus in the chromosome 10, with difference only in 4 bp. This chromosome has been related with the blood pressure regulation (Jacob et al., 1991; Hillbert et al., 1991) and is also associated with different plasma and tissue ACE levels (Kreutz et al., 1995; Ocaranza et al., 2002). The best evidence relating the aforementioned I/D ACE gene polymorphism with cardiovascular risk in humans is the higher risk for developing hypertension in men with allele D (O'Donell et al., 1998; Higaki et al., 2000; Danser and Schunkert, 2000). This epidemiological observation has a recent experimental correspondence in male rats with a similar ACE polymorphism (BB genotype) in which higher ACE is associated with higher Ang II levels and higher levels of chronic hypertension in the Goldblatt model (Ocaranza et al., 2002). This ACE polymorphism in Lewis and Brown Norway rats is associated with circulating Ang II levels as well as plasma and tissue NEP levels (Oliveri et al., 2001). No data are currently available on the relationship of this polymorphism with Ang-(1–7) levels.

We hypothesized here that an ACE gene polymorphism in rats is associated with circulating Ang-(1–7) levels that are inversely related to Ang II plasma levels. This study was also aimed to assess the effect of renovascular hypertension on the Ang-(1–7) levels according to the ACE gene polymorphism.

Material and methods

Animals and experimental model

The experiments were performed following the recommendations of the Guide for the Care and Use of Laboratory Animals (publication NIH No. 85–23, review 1985). Rats were homozygous F_0 (6 weeks of age) and F_2 (4 weeks of age). The F_2 homozygous were obtained after mating male F_0 Brown Norway (BN) with female Lewis strains obtained from Charles Rivers (Willmington, MA, USA). These F_0 rats produced F_1 hybrids that were mated to obtain the F_2 cohort. The animals were given a standard rat chow with salt and water ad libitum. Hypertension was induced using the Goldblatt (Gb) model (2 kidneys—1 clipped) in homozygous F_2 male rats as previously described (Ocaranza et al., 2002; Dussaillant et al., 1996). Animals were sacrificed 4 weeks after surgery. As controls F_2 homozygous rats were sham operated. The animals were given a standard rat chow with salt and water ad libitum. Systolic blood pressure (SBP) was measured blindly throughout the study with the tail cuff method.

ACE polymorphism determination

The polymerase chain reaction (PCR) primers (sense oligonucleotide primer: 5′ATTACCATAGAGGGCAGCAAGATC3′ and the antisense primer: 5′CAGACTTTTCACCAATTTTGACAGC3′) and the procedure used to amplify the microsatellite located at the 5′ end of the intron 13 inside the rat ACE gene and characterized by a (CA)n repeat (Hillbert et al., 1991; Challah et al., 1998). DNA was extracted from circulating leukocytes using Chelex 100 (Walsh et al., 1991).

Plasma ACE activity assay

After decapitation (non-anesthetized animals), trunk blood was rapidly collected into prechilled tubes containing heparin. Plasma ACE activity was measured fluorimetrically using *Z*-phenyl-L-histidyl-L-leucine (Bachem Bioscience Inc, USA) as a substrate and expressed in units per milliliters (1 U=1 nmol L-histidyl-L-leucine formed/min) (Jalil et al., 1991, 1999; Oliveri et al., 2001).

Plasma Ang II and Ang-(1–7) level determination

Blood samples for Ang measurements were rapidly collected after decapitation (non-anesthetized animals) in tubes on ice containing the following inhibitors (0.1 mL of inhibitor solution for 2 mL of blood): 6.25 mM disodium EDTA and 1.25 mM 1,10-phenantroline and 120 μM pepstatin A (Danser et al., 1994; Admiral et al., 1990). These inhibitors were used to prevent Ang I generation, Ang

Table 1
Blood pressure, cardiac weight and plasma ACE in both F_0 genotypes

	Lewis	Brown Norway	
N	8	6	P
BW (g)	259±26	225±26	NS
LVW (mg)	608±50	494±40	NS
SBP (mm Hg)	101±4	108±3	NS
LV/BW	238±11	223±13	NS
Plasma ACE activity (U/mL)	86±11	190±11	<0.05

The data are expressed as mean±SEM. Abbreviations: BW: body weight; LVW: left ventricle weight; SBP: systolic blood pressure; LV/ BW: left ventricle to body weight ratio (mg* 100/ g BW); NS: non-significant.

I to Ang II conversion and Ang I, Ang II and Ang-(1–7) degradation during blood collection and handling of samples. The blood samples were centrifuged at 3000 ×*g* (10 min at 4 °C). Plasma was stored at −80 °C, extracted within 2 days and assayed later. Plasma was directly applied to SepPak cartridges as described below. The cartridges were conditioned with methanol and equilibrated with cold distilled water. Adsorbed Angs were eluted with 2 mL 90% methanol. The methanol was evaporated under vacuum rotation at 4 °C. Angs were separated by reversed-phase HPLC (Nussberger et al., 1986), using a μBondapak C_{18} column (300 × 3.9 mm, 10 μm particle size) and μBondapack C_{18} precolumn. Mobile phase A was 25% methanol, 0.085% H_3PO_4 containing 0.02% NaN_3. Mobile phase B was 75% methanol/0.085% H_3PO_4 containing 0.02% NaN_3. The concentrated SepPak extracts were dissolved in 100 μL mobile phase and centrifuged at 10,000 ×*g* for 5 min before injection. The flow rate was 1.5 mL/min and the working temperature was 45 °C. Elution was performed as follows: 85% mobile phase A—15% mobile phase B from 0 to 5 min, followed by a linear gradient to 40% mobile phase A and 60% mobile phase B until 20 min. Eluates were collected in 0.5 mL fractions in polypropylene tubes containing 20 μl bovine seroalbumin (BSA) 0.1%. Fractions containing Ang II and (1–7) were neutralized with 1 N NaOH.

Ang II was quantified by radioimmunoassay (RIA) using an Ang II antibody kindly donated by Dr A.H.J. Danser (Erasmus University, Rotterdam, The Netherlands). The Ang II antibody has 100% cross-reactivity with Ang II and Ang (4–8), 55% cross-reactivity with Ang III, 73% cross-reactivity with Ang (3–8), and ≤0.2% cross-reactivity with Ang I, Ang (2–10) and with Ang-(1–7). Losses of Ang II during the extraction and HPLC separation of plasma were measured by adding known amounts of radiolabeled Ang II to the tissue prior to homogenization. Ang II recovery from tissue was 65% and from plasma 78%. The coefficients of variation for inter-assay and intra-assay variances were 13% and 6% for Ang II, respectively. The lower limit of detection was 0.4 fmol per fraction for the Ang II assay.

Ang-(1–7) was detected using a polyclonal antibody developed by us in rabbits using conventional methods. The coefficients of variation for inter-assay and intra-assay variances were 4% and 12% for Ang-(1–7), respectively. The range of detection using standards of Ang-(1–7) was between 0.92 and 184 pg/mL.

Statistical analysis

Results are shown as mean±SEM. For F_0 rats comparisons between groups (Lewis and BN) were performed with Student t test for independent measurements, or with the Mann–Whitney test. For F_2 rats one-way ANOVA followed by Student–Newman–Keuls test was used. Two factor ANOVA was also used to assess the effects of both strain and hypertension and their interactions. Linear correlation was also applied. A p value ≤0.05 was considered statistically significant.

Results

ACE genotypes and general characteristics

Genetic variability of ACE was determined by PCR from DNA extracted and purified from circulating leukocytes. ACE microsatellital marker analysis in the F_0 and F_2 cohorts demonstrated that the difference between Lewis rats (with low ACE activity) and BN rats (with high ACE activity) was only in 4 bp (168 and 171 bp, respectively, not shown).

No differences were observed between the Lewis and BN genotypes in both F_0 (Table 1) and F_2 control animals (Table 2) in terms of body weight, LV weight, neither in blood pressure. In the 2 Goldblatt groups, a similar degree of hypertension (about 50% increment) and left ventricular hypertrophy (43% increment) was observed in rats with both genotypes (Table 2).

Plasma ACE activity

Plasma ACE activity was two-fold higher (p<0.05) in both homozygous Brown Norway compared to homozygous Lewis rats in the F_0 as well as in the F_2 normotensive groups (Tables 1 and 2). In the F_2 groups no differences in plasma ACE activity were observed between normotensive and hypertensive groups within each ACE genotype (Table 2). F_0 rats were 4 weeks older than the F_2 generation which explains the small difference in body weight as well as in the LV weight between F_0 rats and sham F_2. However, the

Table 2
Blood pressure, cardiac weight and plasma ACE in the F_2 normotensive and hypertensive groups

	Lewis-S	Lewis-Gb	BN-S	BN-Gb	ANOVA	
N	38	27	36	14	F	P
BW (g)	190±7	184±7	203±5	206±10	1.9	NS
LVW (mg)	476±17	648*±15	512±14	709*±38	29	<0.001
SBP (mmHg)	105±2	167*±5	109±2	160*±5	110	<0.001
LV/BW	251±6	361*±13	246±5	352*±20	40	<0.001
N	17	20	23	10		
Plasma ACE activity (U/mL)	85±10	95±5	192**±10	192**±12	49.2	<0.001

Mean±SEM. Abbreviations: BN: Brown Norway; BW: body weight; LVW: left ventricle weight; SBP: systolic blood pressure; LV/BW (mg*100/g): left ventricle to body weight ratio. Symbols: *p<0.05 vs. their respective sham group (after ANOVA).

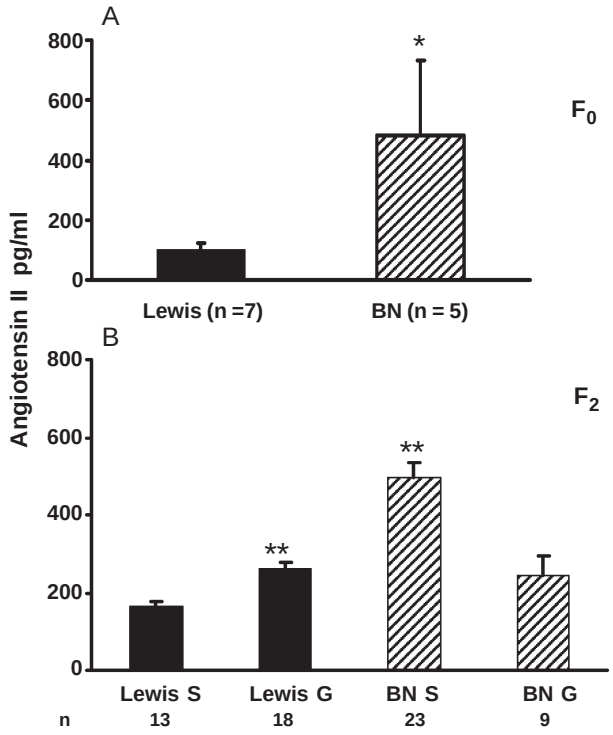

Fig. 1. Plasma Ang II levels in the homozygous normotensive F_0 groups (A) and in the normotensive (sham, S) and hypertensive (Goldblatt, Gb) homozygous F_2 groups (B). Lewis rats are represented in black whereas Brown Norway (BN) rats are represented by hatched bars. Symbols: *$p<0.05$ vs. Lewis; **$p<0.05$ vs. all the other groups (after ANOVA).

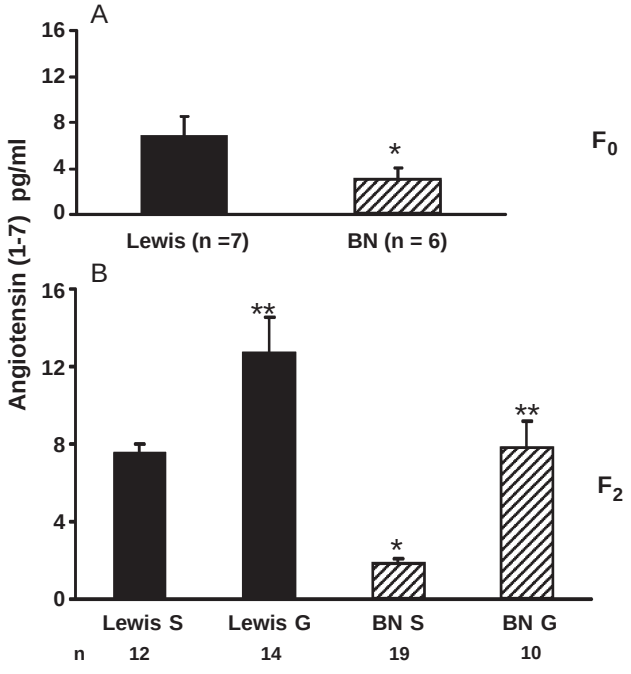

Fig. 2. Plasma Ang-(1–7) levels in the homozygous normotensive F_0 groups (A) and in the normotensive (sham, S) and hypertensive (Goldblatt, Gb) homozygous F_2 groups (B). Lewis rats are represented in black whereas Brown Norway (BN) rats are represented by hatched bars. Symbols: *$p<0.05$ vs. Lewis; **$p<0.05$ vs. sham groups (after ANOVA).

absolute values and the differences in ACE activity between Brown Norway and Lewis strains were the same in both generations (Tables 1 and 2).

Plasma Ang II levels

In the Lewis F_0 rats ($n=7$), plasma Ang II levels were 103 ± 21 pg/mL. In the BN F_0 rats ($n=5$), the levels of plasma Ang II were 400% higher than in the Lewis rats ($p<0.05$; Fig. 1A). In the Lewis F_2 sham rats ($n=13$), plasma Ang II levels were 163 ± 15 pg/mL. In the BN F_2 sham rats ($n=23$), the levels of plasma Ang II were 300% higher than in the Lewis rats ($p<0.05$; Fig. 1B). In the hypertensive BN rats significantly lower Ang II plasma levels were observed compared to their sham operated controls (Fig. 1B).

Plasma Ang-(1–7) levels

In the Lewis F_0 rats ($n=6$), plasma Ang-(1–7) levels were 4.1 ± 0.7 pg/mL. In the BN F_0 rats ($n=6$) the levels of plasma Ang-(1–7) were 87% lower than in the Lewis rats ($p<0.001$;

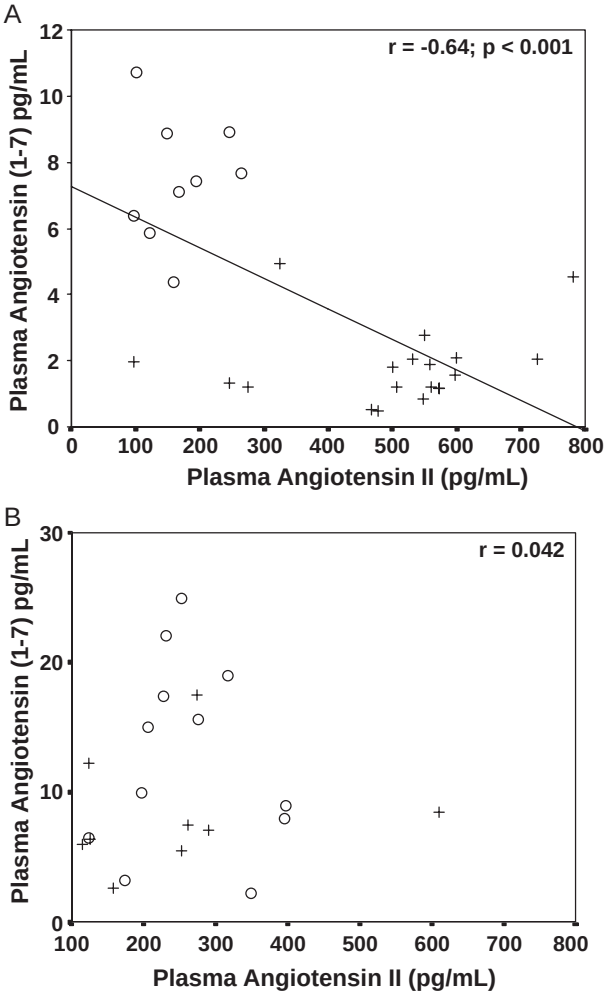

Fig. 3. Relationship between plasma levels of Ang-(1–7) and Ang II in the normotensive (A) and hypertensive (Goldblatt) homozygous F_2 rats (B). Plasma levels of Ang-(1–7) and Ang II were correlated only in the normotensive rats ($r=-0.64$; $p<0.001$). Symbols: o = Lewis rats; + = Brown Norway rats.

Fig. 2A). In the Lewis F_2 rats ($n=12$), plasma Ang-(1–7) levels were 7.6±0.5 pg/mL. In the BN rats ($n=19$), plasma levels of Ang-(1–7) were 75% lower than in the Lewis rats ($p<0.05$; Fig. 2B). Plasma levels of Ang-(1–7) were significantly higher ($p<0.05$) in all renovascular hypertensive rats (F_2) from both genotypes compared with their sham operated animals, however hypertensive Lewis rats still had higher Ang-(1–7) levels than hypertensive BN rats (Fig. 2B). A significant effect of both strain ($F=23$, $p<0.001$) and hypertension on plasma Ang-(1–7) levels ($F=25$, $p<0.001$) was observed without interaction. Plasma levels of Ang II and Ang-(1–7) levels were inversely correlated in the normotensive ($r=-0.64$; $p<0.001$), but not in the hypertensive rats (Fig. 3A–B).

Discussion

The main findings of our study were (a) that this ACE gene polymorphism in the rat is not only associated to ACE and circulating Ang II levels but also to Ang-(1–7) circulating levels, (b) that Ang-(1–7) plasma levels are inversely related to genetically determined Ang II plasma levels and (c) that plasma levels of Ang-(1–7) increase in this model of renovascular hypertension, independently of the ACE genotype.

We examined here two ACE genotypes in the rat. The BN and Lewis rats exhibit contrasted levels of circulating and tissue ACE and NEP. This degree of genetic determination of ACE expression in inbreed rat strains offers an opportunity to assess the interaction between genetic and environmental determinants of ACE and Ang-(1–7).

Previous identification of a microsatellite marker in the rat ACE gene, specifically in the intron 13 on the chromosome 10, has allowed differentiation of ACE alleles among different rat strains (Jacob et al., 1991; Hillbert et al., 1991) and their association to different levels of plasma ACE [20]. In rats, ACE activity in cultured vascular cells and the neointima formation in the carotid artery after balloon injury are influenced by the level of ACE expression (Challah et al., 1998). Similar and consistent findings in terms of plasma ACE, Ang II and Ang-(1–7) levels were observed here in both homozygous F_0 and F_2 generations (Figs. 1 and 2).

Ang-(1–7) is formed mainly from Ang I. Two of the endopeptidases forming Ang-(1–7), neutral endopeptidase 24.11 and metalol endopeptidase 24.15 have been implicated in the metabolism of vasodilator peptides such as bradykinin and atrial natriuretic factor. We observed previously that plasma and tissue ACE activities were significantly higher in BN than in Lewis rats whereas NEP activity in Lewis rats was higher in the serum and tissue than in BN rats [26]. Higher NEP activity–genetically determined–seems to explain increased Ang-(1–7) levels observed here in the Lewis rats (and vice versa in the BN rats). In the aforementioned experiments, plasma ACE activity was inversely correlated with serum and lung NEP activities (aortic ACE and NEP activities were also inversely correlated) (Oliveri et al., 2001). Thus, genetically determined higher ACE expression in rats–as well as in humans–is inversely related to NEP activity, which probably will be also associated with lower Ang-(1–7) tissue levels (and vice versa) (Oliveri et al., 2001; Jalil et al., 2004). There are no data on the role of ACE2 in determining Ang-(1–7) levels in these rats with genetically determined ACE expression.

To better understand the previously observed mechanism of ACE modulation on NEP activity we undertook this study, determining Ang II and Ang-(1–7) levels in this genetic rat model of differential ACE activity, which is not simple. There are no available data on circulating levels of Ang-(1–7) in related or similar genetic models.

Ang-(1–7) has been found distributed throughout cardiac myocytes in healthy cardiac tissue of the Lewis rat strain. Moreover, the remodeling of cardiac tissue 4 weeks after coronary artery ligation seems to be associated with loss of Ang-(1–7) immunoreactivity within the area of the infarct and an apparent increased expression of the peptide in the zones bordering the infarcted region of the left ventricle suggesting that Ang-(1–7) is present in significant quantities in the myocardium, a finding that suggests local synthesis of the heptapeptide (Ferrario, 2002).

ACE–genetically determined in a significant extent–is responsible for the degradation of Ang-(1–7) into the inactive fragment Ang-(1–5). Ang-(1–7) may act as an endogenous inhibitor of the N-domain of the somatic form of ACE (Deddish et al., 1998). Thus, long-term administration of ACE inhibitors results in significant elevations in plasma and urinary concentration of Ang-(1–7) in animals and humans (Chappell and Ferrario, 1999; Luque et al., 1996), whereas blockade of Ang-(1–7) receptors or inhibition of Ang-(1–7) activity partially reverses the antihypertensive effect of long-term administration of lisinopril alone or in combination with losartan (Iyer et al., 1998). Our current results are consistent with this rational (higher ACE due to an ACE polymorphism generates higher Ang II and lower Ang-(1–7) levels, and vice versa).

Circulating Ang II levels should be also genetically influenced by an ACE polymorphism. In this regard, no association of this polymorphism with plasma Ang II levels has been found in humans neither in rats (Lachurié et al., 1995; Challah et al., 1998). In normotensive males, the DD genotype was associated with an enhanced blood pressure rise induced by Ang I infusion (Ueda et al., 1995; van Dijk et al., 2000). Our results show that plasma Ang II levels were 50% higher in the homozygous BN rats than in the Lewis rats, which was also previously observed by us (Ocaranza et al., 2002). Reasons explaining differences between our results and other reports are probably due to methodological differences since determination of Ang II–as well as of Ang-(1–7)–is difficult and time consuming. In this context, our Ang II and Ang-(1–7) levels in the Lewis F_0 rats are different compared to a previous report (Ishiyama et al., 2004), which might be due to methodological differences (we use HPLC and RIA whereas Ishiyama et al. use only RIA). However, in the present study, we have observed a very similar and consistent response in plasma ACE activity and levels of Ang II to what we observed previously 9 weeks after surgery in the same strains: (1) No modification in plasma ACE activity in the Goldblatt rats from both strains and (2)

increased Ang II plasma levels only in the Lewis Gb rats (rats with higher baseline levels of Ang-(1–7).

Bradykinins, not measured here, could have been different in both genotypes (possibly higher levels in Lewis rats) since bradykinin levels are determined by ACE. In this regard, a longer half-life of serum bradykinins in humans with the II genotype, with genetically higher levels of plasma ACE has been observed (Brown et al., 1998). Moreover, it has been observed recently in humans that the ACE D allele has a significant effect on the in vivo degradation of BK, and that the ratio of bradykinin-(1–5) in response to BK infusion is significantly higher in the presence of the D allele (Murphey et al., 2000).

In animal studies, Ang-(1–7) exerts physiological and pharmacological effects such as vasodilation (Brosnihan et al., 1998; Li et al., 1997), inhibition of protein synthesis (Freeman et al., 1996), and natriuresis (Handa et al., 1996). Ang-(1–7) also preserves cardiac function and improves coronary perfusion and aortic endothelial function in an experimental model of heart failure produced by ligation of the left coronary artery in the rat (Loot et al., 2002). An antitrophic effect of Ang-(1–7) has also been observed in a rat model of vascular injury, in which a 12-day infusion of Ang-(1–7) prevented neointima proliferation after endothelial denudation of a carotid artery (Strawn et al., 1999). Thus, Ang-(1–7) may buffer the vasoconstrictor and growth promoting actions of Ang II and possibly there is a cardiovascular protective role of Ang-(1–7) under conditions in which there is an activation of the renin–angiotensin system (Ferrario, 2002). The vasodilator actions of Ang-(1–7) depend on an as yet not fully characterized intracellular signaling mechanism that may depend on secretion of prostacyclin, release of nitric oxide, or amplification of the vasodilator effects of bradykinin, alone or in combination (Ferrario and Iyer, 1998).

The physiological effects of Ang-(1–7) plus the fact that Ang-(1–7) levels are lower in BN rats (as well as in hypertensive humans with the ACE DD genotype, Jalil et al., 2003) could also explain why genetically determined higher ACE expression in BN male rats enhances chronic hypertensive response after the induction of renovascular hypertension as was observed previously (Ocaranza et al., 2002) and could also explain the higher risk for hypertension observed in men with the D allele (O'Donell et al., 1998; Higaki et al., 2000; Danser and Schunkert, 2000). However, despite the differences between the two strains in plasma Ang-(1–7) and Ang II levels observed here (the relationship between Ang II and Ang-(1–7) was >150 in the BN and less than 25 in the Lewis rats), no differences with regard to blood pressure and LVW were found. Based on our current findings as well on our previous data in the same model (Ocaranza et al., 2002) we conclude that Ang-(1–7) has no role in LVH in this hypertensive model. The current data do not support either a significant role of Ang-(1–7) in the blood pressure response in this model.

On the other side, a general protective mechanism of increased plasma Ang-(1–7) levels–independent of the ACE genotype–in experimental renovascular hypertension seems reasonable. Some data support the hypothesis that the hemodynamic effects of neurohormonal activation after salt restriction in rats stimulate a tonic depressor action of Ang-(1–7) (Iyer et al., 2000). Further studies replicating our observation will support this preliminary finding. There are studies in experimental hypertension in which the role of Ang-(1–7) has been evaluated by blocking Ang-(1–7) (Bayorth et al., 2002). Recently, increased levels of Ang-(1–7) were observed in children with renovascular and with essential hypertension (Simoes et al., 2004).

In conclusion, there is an inverse relationship between circulating Ang II and Ang-(1–7) in rats associated with an angiotensin I converting enzyme gene polymorphism. This inverse relation could be due to genetically determined higher ACE activity (as well as to lower NEP activity) in the BN rats and could also be the case in humans with the D allele. Besides, plasma levels of Ang-(1–7) increase in renovascular hypertension, independently of the ACE genotype, which may contribute to balance the effects of hypertension on cardiovascular function and remodeling.

Acknowledgments

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