Angiotensin I-converting enzyme gene polymorphism influences chronic hypertensive response in the rat Goldblatt model

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Background and objective **In humans, the insertion/deletion polymorphism in the angiotensin (Ang) I converting enzyme (ACE) gene significantly determines ACE activity. The deletion allele induces higher ACE levels and is associated with hypertension in men. In the rat, a microsatellite marker in the ACE gene allows differentiation of the ACE alleles among strains with different ACE levels. We evaluated the effect of genetically determined ACE expression on the development of renovascular hypertension in the rat.**

Methods and results **Systolic BP (SBP), ACE and angiotensin II (Ang II) levels were measured using the Goldblatt (Gb) model (two kidneys, one clip) in homozygous males of two inbred strains (F_2) of Lewis × Brown-Norway (BN) rats. SBP was significantly higher in the BN-Gb rats compared to the Lewis-Gb rats throughout the study ($F = 239.6$, $P < 0.001$). An interaction was observed between SBP and strain ($F = 2.92$, $P < 0.01$). Plasma ACE activity was 100% higher in the BN-Gb than in the Lewis-Gb rats ($P < 0.05$). Ang II plasma levels were higher in the BN-sham than in the Lewis-sham rats (255 ± 22 versus 161 ± 16 pg/ml, $P < 0.05$), increased in both Gb groups and correlated significantly with SBP ($r = 0.58$, $P < 0.01$).**

Conclusions **Genetically determined ACE expression in male rats enhances the chronic hypertensive response after the induction of renovascular hypertension. A relationship between circulating Ang II and the development of hypertension was also observed in this experimental model of genetically modulated hypertension.**

Keywords: angiotensin, angiotensin I-converting enzyme, genetics, hypertension, Brown-Norway rat, Lewis rat, Goldblatt model

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Introduction

Angiotensin I-converting enzyme (ACE) converts the decapeptide angiotensin I (Ang I) to the vasoconstrictor octapeptide angiotensin II (Ang II), degrades bradykinin (BK) and may decrease the clearance of angiotensin 1–7 (Ang 1,7) [1,2]. In this way, ACE activity contributes to determination of the circulating and tissue levels of Ang II and to modulation of vascular tone and arterial blood pressure.

In different populations an insertion/deletion (I/D) polymorphism of the ACE gene determines almost half the variance of circulating ACE [3–5]. Higher levels of circulating ACE are associated with the presence of the deletion allele. In humans, this polymorphism could be a marker for a closely linked but unidentified sequence variant that modulates the expression of the ACE gene in such a way that the deletion allele is associated with higher ACE activity in plasma [3], in T lymphocytes [6] and in the heart [7].

Previous identification of a microsatellite marker in the rat ACE gene, specifically in intron 13 on chromosome 10, has allowed differentiation of ACE alleles among different rat strains [8,9] and their association with different levels of plasma ACE [10]. In rats, Challah *et al.* [11] observed that ACE activity in cultured vascular cells and the neointima formation in the carotid artery after balloon injury were influenced by the level of ACE expression. In that study no effect of the ACE polymorphism was observed on plasma Ang II levels, nor on acute response of BP to intravenous Ang I [11]. The effect of the ACE polymorphism on chronic blood pressure modulation, and whether there is a relationship with Ang II levels, is still controversial.

The aim of this study was to assess the effect of genetically determined ACE expression on the development of hypertension in male rats of two inbred strains with different ACE expression and activity after the induction of renovascular constriction. We also assessed whether these responses were associated with changes in plasma or left ventricular (LV) ACE activity and Ang II levels.

Methods

Experimental protocol

The experiments were approved by the Research Commission from the Medical School, P. Catholic University of Chile. Male normotensive rats (body weight 80–100 g, $n = 117$) were used. They were homozygous F_2 after mating male F_0 Brown-Norway (BN) with female Lewis (L) strains obtained from Charles Rivers (Willmington, Massachusetts, USA) to obtain a more homogeneous background. These F_1 hybrids were mated to obtain the F_2 cohort. In these F_2 rats, the polymerase chain reaction (PCR) was used to amplify the microsatellite located at the 5′ end of intron 13 of the rat ACE gene characterized by a $(CA)_n$ repeat [9,11].

Hypertension was induced using the Goldblatt (Gb) model (two kidneys, one clip) as described previously [12,13]. 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, using the tail-cuff method.

Nine weeks after surgery the unaesthetized rats were decapitated and all the blood collected. Hearts were quickly removed, and freed from atrial tissue. The aorta, pulmonary artery and ventricles were carefully separated with small scissors and washed with cold saline solution. Left ventricular hypertrophy (LVH) was quantified by the relative left ventricular weight (RLVW = LV × 100/body mass).

ACE gene polymorphism determination

The ACE polymorphism was determined in DNA extracted from circulating leucocytes and amplified by PCR. One blood sample obtained from the marginal vein in the tail was resuspended in deionized sterile water and incubated at room temperature. The blood was then centrifuged, the supernatant was eliminated and the pellet was resuspended in 200 µl of 5% Chelex-100 [14], (Gibco BRL, New York, USA), and incubated at 56°C for 30 min. The mixture was then incubated at 100°C and vortexed. Finally, the DNA was obtained after centrifugation at 15 000 *g*. DNA purity and concentrations were determined in agarose gels (1.5%).

Extracted DNA was amplified by PCR according to Hilbert *et al.* [9]. The reaction mixture contained 20 µl of DNA (50–200 ng), PCR buffer 1X (Tris, 20 mmol/l; KCl, 50 mmol/l); $MgCl_2$, 1 mmol/l; dNTPs, 100 µmol/l (Gibco BRL, New York, USA); *Taq* polymerase, 1 UI (Gibco BRL, New York, USA), the sense primer: 5′ ATTACCATAGAGGGCAGCAAGATC 3′ (10 pmol) and the antisense primer: 5′ CAGACTTTTCAC CAATTTTGACAGC 3′ (10 pmol). The DNA amplification cycles were: one cycle at 94°C, then 30 cycles, each of 30 s at 94°C, 30 s at 65°C and 30 s at 72°C. One final cycle at 72°C was performed. The antisense primer was radiolabelled with [^{32}P]ATP, according to Richardson [15]. Amplification products were resolved by acrylamide–bisacrylamide electrophoresis gels (6%) [8]. Finally, the gel was radioautographed and developed.

ACE activity assay

Plasma ACE activity was measured fluorimetrically using Z-phenyl-L-histidyl-L-leucine (Bachem Bioscience Inc, USA) as a substrate [16–19] and expressed in U/ml (1 U =1 nmol L-histidyl-L-leucine formed/min). For measurement of tissue ACE activity, 100 mg of LV were homogenized with buffer (Tris–HCl, 50 mmol/l, pH 8.0; NaCl 1%). The extract was centrifuged at 4°C and the supernatant was recentrifuged at 15 000 *g* (60 min at 4°C). The pellet was resuspended in buffer and centrifuged at 15 000 *g* (5 min at 4°C). Finally, the pellet was resuspended in Tris–HCl (50 mmol/l, pH 8, 1% NaCl) and 8 mmol/l 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS). Protein concentration was measured according to Bradford [20]. Tissue ACE activity was expressed in U/mg protein.

Plasma and LV Ang II level determination

Blood samples for Ang measurements were collected rapidly after decapitation (non-anaesthetized animals) in glass tubes (on ice) containing the following inhibitors (0.1 ml of inhibitor solution for 2 ml of blood): 6.25 mmol/l disodium EDTA and 1.25 mmol/l 1,10-phenantroline. These inhibitors were used to prevent Ang I generation, Ang I to Ang II conversion and Ang I and II degradation during blood collection and handling of samples [21]. 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 within 2 weeks. Plasma was directly applied to SepPak cartridges as described below. Hearts were quickly removed and the ventricles were excised and immediately frozen in liquid nitrogen and stored at −80°C until assay. LVs were homogenized with an Ultraturrax in an ice-cold extraction solution (80% ethanol, 0.1 N HCl). Homogenates were centrifuged at 20 000 *g* at 4°C, and the supernatants concentrated to 5 ml by evaporation. The remainder of each supernatant was diluted in 0.1% H_3PO_4 and recentrifuged at 20 000 *g* (20 min at 4°C).

The supernatant was diluted with 0.02% H_3PO_4 and concentrated by reversible adsorption to octadecylsilyl silica cartridges (SepPak C-18, Waters, Milford, Massachusetts, USA). The cartridges were conditioned with methanol and equilibrated with cold distilled water. Samples were passed through the cartridge at 4°C, followed by washes with cold water and hexane (tissue samples). Adsorbed angiotensins (Angs) were eluted with 2 ml 90% methanol. The methanol was evaporated under vacuum rotation at 4°C. Angs were separated by reversed-phase high-performance liquid chromatography (HPLC), according to Nussberger *et al.* [22], 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 Sep-Pak 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 serum albumin (BSA) 0.1%. Fractions containing Ang II 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 Universität, Rotterdam, The Netherlands). This 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 homogenization, extraction and HPLC separation of plasma and tissues were measured by adding known amounts of radiolabelled Ang II to the tissue prior to homogenization. Ang II recovery from tissue was 65% and from plasma 78% ($n = 15$). The coefficients of variation for inter-assay and intra-assay variances were 13 and 6.5% for Ang II, respectively. The lower limit of detection was 0.4 fmol per fraction for the Ang II assay.

Plasma renin activity

This was measured in duplicate using the GammaCoat® [^{125}] Plasma Renin Activity RIA kit (DiaSorin, Minnesota, USA).

Statistical analysis

Results are shown as mean ± SEM. Comparisons were performed with two-way ANOVA using strain and the experimental procedure (sham or Gb) as factors. Subsequent Student–Newman–Keuls tests were used as *post hoc* tests. The systolic blood pressure during the whole study as a function of time, strain and the experimental procedure (sham or Gb), as well as their interactions, was analysed with multivariate ANOVA for repeated measurements (General Linear Model, SPSS 8.0). Systolic blood pressure at 4 and 9 weeks was also analysed with two-way ANOVA (using strain and experimental procedure as factors). Linear correlation and multivariate regression analysis was also utilized. A P value ⩽ 0.05 was considered statistically significant.

Results

ACE polymorphism, plasma and LV activities

Figure 1 depicts the acrylamide electrophoresis results of the amplified fragments in the F_2 BN and Lewis rats. As external controls for the polymorphic variants, DNA from BN and Lou rats (generously provided by Mireille Challah, Inserm U367, France) was used (data not shown). A clear difference in both homozygous genotypes was observed, accounting for a difference in 4 bp between both genotypes.

As expected, plasma and LV ACE levels were significantly ($P < 0.05$) increased in both sham and Goldblatt BN groups compared with the Lewis rats (Table 1, $n = 40$). Plasma and LV ACE activities were significantly correlated ($r = 0.58$, $P < 0.01$).

Body weight and LV mass

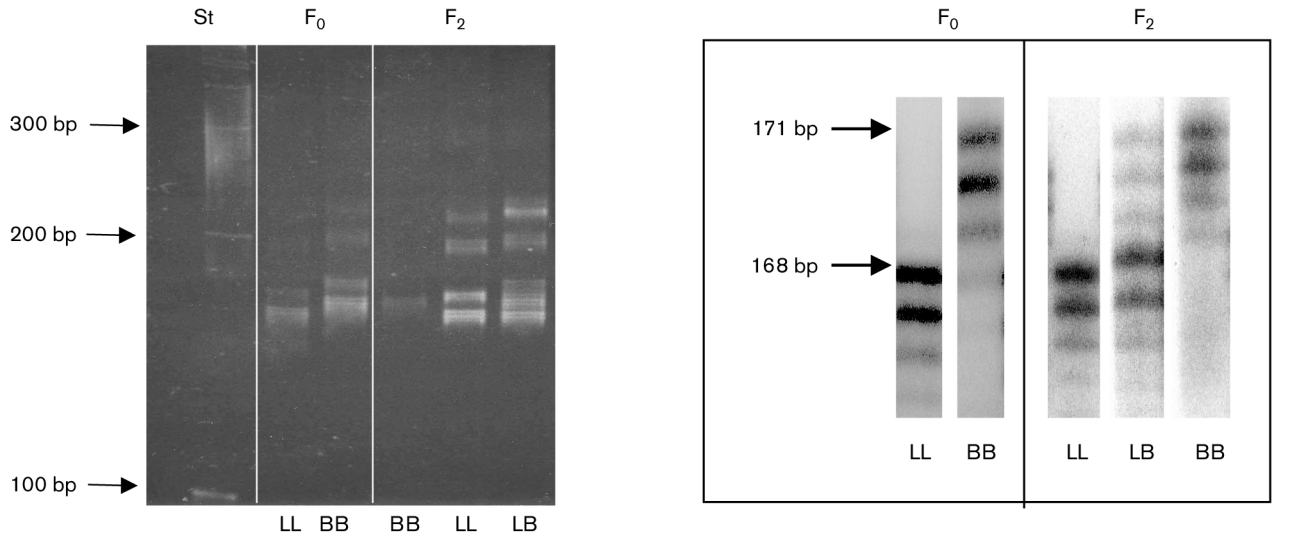
As shown in Table 2, 9 weeks after surgery there were no differences in the body weight among the four experimental groups ($n = 117$). In both Gb groups a significant degree of LVH was observed (18–30% compared with their respective sham-operated groups). The degree of LVH, assessed by LVW/BW, was larger in the Gb BN animals than the Gb Lewis animals. However, no interaction was found between strain and the experimental procedure (sham or Gb) regarding LVW/BW (data not shown). No increased right ventricular weight (RVW) was observed in Gb groups.

Systolic blood pressure

Increased systolic blood pressure (SBP) was observed from the first week after surgery in both Gb groups (Fig. 2). From 2 weeks after surgery the level of hypertension was significantly higher in the BN Gb rats compared to the respective Lewis Gb rats, and remained consistently so until the end of the study, 9 weeks after surgery ($F = 239.6$, $P < 0.001$). A significant interaction on the effect of hypertension was observed between the strain and the experimental procedure (sham or Gb, $F = 2.92$, $P = 0.005$; Fig. 2 and Table 3, $n = 117$), meaning higher SBP increments as a function of time in the BN Gb group compared with the Lewis Gb rats. SBP was significantly correlated with the LV/body weight ratio ($r = 0.56$, $P < 0.001$, not shown).

No correlation was found between plasma ACE activity and SBP ($r = 0.06$, $n = 39$), nor with LV mass

Fig. 1



Characterization of angiotensin converting enzyme (ACE) gene polymorphism by a microsatellite marker in F_0 and F_2 progenies. DNA was isolated from blood and a specific ACE gene promotor region was amplified by polymerase chain reaction (PCR) using sense and [^{32}P]-labelled antisense oligonucleotides. Amplified DNA fragments were separated by polyacrylamide gel electrophoresis and the gels were radioautographed for 16 h at −80°C. L and B are Lewis and Brown-Norway ACE gene alleles, respectively. Left arrows show the molecular weight ladder.

Table 1 **Angiotensin I converting enzyme activity in the different experimental groups ($n = 40$, mean ± SEM)**

	Lewis, sham	Lewis, Goldblatt	BN, sham	BN, Goldblatt	Two-way ANOVA (F and P values)		
n	12	11	9	8	Strain	Exp proc	Interaction
Plasma (U/ml)	108 ± 5	99 ± 4	242 ± 16*#	198 ± 14*	136.9, < 0.01	7.29, < 0.05	No
LV (U/mg prot)	0.61 ± 0.08	0.60 ± 0.13	1.48 ± 0.10*	1.08 ± 0.31*	17.6, < 0.01	1.52, > 0.1	No

Abbreviations: BN, Brown Norway; LV, left ventricle; Exp proc, experimental procedure (e.g. sham or Goldblatt). * $P < 0.05$ versus Lewis groups (after ANOVA); #$P < 0.05$ versus BN Goldblatt (after ANOVA).

Table 2 **Body weight and cardiac mass ($n = 117$, mean ± SEM)**

	Lewis, sham	Lewis, Goldblatt	BN, sham	BN, Goldblatt	Two-way ANOVA (F and P values)		
n	41	25	32	19	Strain	Exp proc	Interaction
BW (g)	258 ± 7	275 ± 9	276 ± 7	252 ± 1	0.09, > 0.1	0.15, > 0.1	No
LVW (mg)	542 ± 17	719 ± 34*	578 ± 15	761 ± 5*	2.05, > 0.1	42.95, < 0.01	No
RVW (mg)	154 ± 6	158 ± 6	167 ± 8	154 ± 9	0.29, > 0.1	0.28, > 0.1	No
LV/BW (mg 100/BW)	211 ± 5.6	268 ± 15*	212 ± 5.2	303 ± 20*,#	2.82, > 0.1	47.82, < 0.01	No
LVW/RVW	3.7 ± 0.2	4.6 ± 0.2*	3.6 ± 0.1	4.9 ± 0.2*	0.44, > 0.1	38.75, < 0.01	No

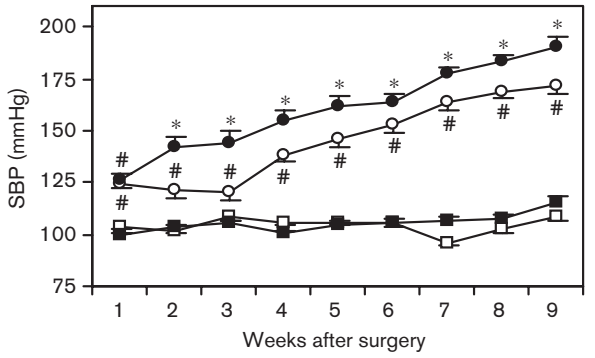
BN, Brown-Norway; BW, body weight; Exp proc, experimental procedure (e.g. sham or Goldblatt); LVW, left ventricular weight; RVW, right ventricular weight; LV, left ventricle; RV, right ventricle. *$P < 0.05$ versus sham groups; #$P < 0.05$ versus Lewis Goldblatt (after ANOVA).

($r = -0.016$). Neither was LV ACE activity correlated with LV mass ($r = 0.042$).

Ang II concentrations and plasma renin activity

Ang II plasma levels at the end of the experiments ($n = 28$) were significantly higher ($P < 0.05$) in the sham BN rats than in the sham Lewis rats (Table 4). In the Lewis Gb group the levels of plasma Ang II were significantly increased compared to the sham Lewis group ($P < 0.05$). In the BN animals the difference in the levels of plasma Ang II between the Gb and sham rats did not reach statistical significance. Ang II plasma levels were significantly correlated with SBP, as depicted in Figure 3 ($r = 0.58$, $P < 0.01$, $n = 28$) but not

Fig. 2



Time course of systolic blood pressure (SBP, mean ± SEM) in the four experimental groups. Symbols: ● = Brown-Norway Goldblatt ($n = 19$), ○ = Lewis Goldblatt ($n = 25$), ■ = Brown-Norway sham ($n = 32$) and □ = Lewis sham ($n = 41$). * $P < 0.05$ versus Lewis Goldblatt and both sham groups; #$P < 0.05$ versus both sham groups (ANOVA for repeated measurements $F = 239.6$, $P < 0.001$). A significant interaction on the effect of hypertension was observed between the strain and the experimental procedure (sham or Goldblatt, $F = 2.92$, $P = 0.005$) meaning higher SBP increments as a function of time in the Brown-Norway Goldblatt group compared with the Lewis Goldblatt rats.

with LV weight nor with LVW/BW ratio ($r = 0.12$ and 0.12, respectively).

No differences were observed in the LV Ang II concentrations among the four groups. Plasma and LV Ang II levels were not correlated ($r = 0.15$).

Plasma renin activity (PRA) was higher in both Brown-Norway groups (Table 4, $n = 42$) and was correlated with plasma angiotensin II levels ($r = 0.48$, $P < 0.02$) and with SBP ($r = 0.32$, $P < 0.05$).

By multivariate regression analysis, both SBP and PRA had independent effects on LVH. Based on the beta-values, the effect of SPB on LVH was mildly larger than the effect of PRA (0.433 and 0.403, respectively).

Discussion

The main findings of this study were: (1) the ACE polymorphism was associated with an increased chronic hypertensive response after the induction of renovascular stenosis in rats and (2) a direct relationship between circulating Ang II and hypertension was observed in this experimental model of genetically modulated renovascular hypertension.

In humans, the I/D ACE polymorphism is correlated with differences in plasma levels of ACE in healthy people, accounting for 47% of the total phenotypic variance of plasma ACE [2]. A similar genetic determination of ACE activity has been observed previously in rats [10] and was confirmed in the present study. In addition, in the human heart, ACE activity is higher in subjects with the DD ACE genotype than in subjects with the ID and the II ACE genotypes [7]. We observed here increased LV ACE activity in rats with the BN genotype compared with the Lewis genotype.

Based on comparative responses to acetylcholine and nitroprusside in the vessels of hypertensive subjects, it has been suggested that homozygosity for the D allele is characterized by significant reduction in endothelium-dependent vasorelaxation compared to the ID ACE genotype [23]. In subjects without hypertension, ischaemic heart disease or diabetes, higher plasma ACE activity has been associated with increased carotid wall thickening [24]. Thus, this polymorphism could also affect vascular structure and responses to different functional stimuli.

In general, no relation has been found between BP and the I/D ACE polymorphism in human studies [25,26]. However, in two recent, large, population-based studies, the D allele was associated with diastolic BP and hypertension only in men [27,28]. Furthermore, in hypertensive Italian subjects, the DD ACE genotype was associated with higher ambulatory SBP [29]. More recently, a significant association has been observed between this polymorphism and salt-sensitive hypertension. Patients with the II and ID genotypes have higher prevalence of salt sensitivity than the DD hypertensives [30].

One confounding factor when assessing the relationship between the I/D ACE polymorphism and hypertension in the clinical setting is the difficulty in determining the onset of hypertension with any precision. This was

Table 3 **Systolic blood pressure 4 and 9 weeks after surgery ($n = 117$, mean ± SEM)**

	Lewis, sham	Lewis, Goldblatt	BN, sham	BN, Goldblatt	Two-way ANOVA (F and P values)		
n	41	25	32	19	Strain	Exp proc	Interaction
SBP_4 (mmHg)	106 ± 2	138 ± 3*	101 ± 1	154 ± 6*,#	4.7, < 0.05	268, < 0.01	17.3, < 0.01
SBP_9 (mmHg)	108 ± 2	172 ± 4*	115 ± 3	191 ± 4*,#	19.4, < 0.01	547, < 0.01	4.1, < 0.05

Abbreviations: BN, Brown-Norway; Exp proc, experimental procedure (sham or Goldblatt). *$P < 0.05$ versus sham; #$P < 0.05$ versus Goldblatt Lewis (after ANOVA).

Table 4 **Angiotensin II levels ($n = 28$) and plasma renin activity ($n = 42$) at the end of the experiments (mean ± SEM)**

	Lewis, sham	Lewis, Goldblatt	BN, sham	BN, Goldblatt	Two way ANOVA (F and P values)		
n	6	7	7	8	Strain	Exp proc	Interaction
Plasma Ang II (pg/ml)	161 ± 7	248 ± 35*	260 ± 7*	299 ± 11*	15.32, < 0.01	10.75, < 0.01	No
LV Ang II (pg/mg LV)	0.91 ± 0.14	0.73 ± 0.23	1.15 ± 0.15	0.63 ± 0.11	0.18, > 0.1	4.49, 0.045	No
n	11	8	12	11			
PRA (ng/ml/h)	5.4 ± 0.9	5.2 ± 0.7	7.7 ± 1.2**	10.2 ± 3**	7.09, < 0.01	1.66, > 0.1	No

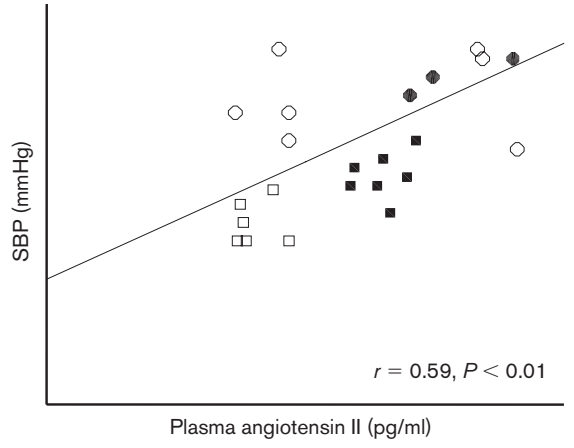
Abbreviations: BN, Brown-Norway; Exp proc, experimental procedure (e.g. sham or Goldblatt); Ang II, angiotensin II; PRA, plasma renin activity.
*$P < 0.05$ versus Lewis sham; **$P < 0.05$ versus both Lewis groups (after ANOVA).

one reason for performing this experimental study, where the onset of the stimulus for development of hypertension was totally controlled.

Other reasons assumed in the literature to explain the absence of relationship between the I/D ACE polymorphism and hypertension in humans is that, despite its association with major differences in plasma ACE levels, this polymorphism has not been found to modify the plasma levels of Ang II, aldosterone or renin [26]. In normotensive subjects this polymorphism did not modify BP increase induced by Ang I infusion [26], but the opposite has also been observed in two other studies [31,32].

In this experimental study, the Ang II plasma levels were 50% higher in the BN sham rats compared to the Lewis sham animals, an effect not observed in earlier studies of this experimental model [11]. This difference could be due to intrinsic differences between both rat strains. Therefore, a baseline difference in the levels of this vasoconstrictor peptide exists between these two genotypes in the rat, before the onset of the hypertensive stimulus. This difference in plasma Ang II – due to the ACE polymorphism – does not explain baseline BP, but it may be able to modulate the BP response to this hypertensive procedure, which induces higher plasma Ang II levels in the early stages [33].

Fig. 3



Linear regression depicting the relationship between plasma angiotensin II and systolic blood pressure (SBP) 9 weeks after surgery. Symbols: ● = Brown-Norway Goldblatt ($n = 8$), ○ = Lewis Goldblatt ($n = 7$), ■ = Brown-Norway sham ($n = 7$) and □ = Lewis sham ($n = 6$).

We cannot rule out that additional mechanisms explaining the enhanced hypertensive response in the BN genotype may be due to reduced circulating BK and/or Ang 1–7 levels (or other vasodilators) after renovascular stenosis. In studies performed comparatively in BN and Lou rats, no different responses in BP were observed after injecting either Ang I or BK [11]. However, 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 [34]. The present observations suggest that increased circulating Ang II – genetically modulated – is a major factor for differences in developed renovascular hypertension.

Since this study was performed only in male rats, we cannot be totally sure that this observation is relevant only to males. In men, renin and prorenin are approximately 30 and 50% higher, respectively, than in women [35]. Renin is the second major enzyme of the system and is responsible for generation of the substrate for ACE. It may be speculated that males with genetically elevated ACE levels are confronted with higher Ang I levels [36] and, thereby, may have higher Ang II levels, as was observed in this study. Our plasma renin activity data are different from those of Challah *et al.* [11], who did not find significant differences in plasma renin activity between male BN and Lou rats, but are consistent with our observed levels of plasma Ang II. The difference between these two circulating parameters in our study is that both the ACE polymorphism and the 9-week Gb procedure influenced plasma Ang II levels, whereas plasma renin activity was influenced

only by the ACE polymorphism (Table 4). In our study, renin was more related to the Ang II levels and SBP than ACE activity.

In our study no effect of higher levels of hypertension in the BN Gb rats was observed on the degree of left ventricular hypertrophy (LVH). This observation is consistent with most of the negative human studies relating the D allele with LVH in hypertension [37,38] and also with a recent experimental study by Perry *et al*. [39] showing that mice with one and two ACE genes develop a similar amount of cardiac hypertrophy and elevation of LV Ang II after aortocaval fistula.

In humans, the I/D ACE polymorphism might induce differential responses to physiological or pathological vascular stimuli. This concept has been suggested by observations in rats with genetically determined high levels of ACE which develop higher neointimal proliferation in response to carotid injury [11]. In humans, some observations also support this notion, such as a higher re-stenosis rate after coronary stenting in patients with the D allele [40,41], increased LV mass in response to exercise in subjects with the D allele [42] and the development of early atherosclerosis in carotid arteries in the hypertensive population [43].

In conclusion, genetically induced increased ACE expression enhances the chronic hypertensive response to renovascular stenosis in male rats. In addition, higher levels of circulating Ang II were positively correlated with the development of hypertension in this experimental model of genetically associated hypertension.

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