# Increased Aortic NADPH Oxidase Activity in Rats With Genetically High Angiotensin-Converting Enzyme Levels

Jorge E. Jalil, Alfonso Pérez, María Paz Ocaranza, Jorge Bargetto, Alfonso Galaz, Sergio Lavandero

***Abstract***—In humans and rats, angiotensin I–converting enzyme activity is significantly determined by a gene polymorphism. Homozygous Brown Norway rats have higher plasma angiotensin I–converting enzyme activity and circulating angiotensin II (Ang II) levels than Lewis rats. Because Ang II induces NAD(P)H oxidase activation, we hypothesized here that Brown Norway rats have higher vascular NAD(P)H oxidase activity and superoxide anion production than Lewis rats. Homozygous Brown Norway (n=15) and Lewis (n=13) male rats were used. Plasma angiotensin I–converting enzyme activity (by fluorimetry), Ang II levels (by high-performance liquid chromatography and radioimmunoassay), and aortic NAD(P)H oxidase activity, as well as superoxide anion production (by chemiluminescence with lucigenin) were measured. Plasma angiotensin I–converting enzyme activity and Ang II levels were 100% higher in Brown Norway rats than in Lewis rats ($P<0.05$). Aortic angiotensin I– converting enzyme, but not Ang II, was elevated ($P<0.05$). Aortic superoxide anion production and NAD(P)H oxidase activity were 300% and 260% higher in Brown Norway than in Lewis rats, respectively ($P<0.05$), which was not observed in Brown Norway rats treated with candesartan (10 mg/kg per day for 7 days). Endothelial NO synthase activity in the aorta from Brown Norway rats was significantly lower than in Lewis rats. However, inducible NO synthase activity and both endothelial NO synthase and inducible NO synthase mRNA and protein levels were similar in both genotypes. In summary, Brown Norway rats have higher vascular NAD(P)H oxidase activity and superoxide anion production than Lewis rats, suggesting the presence of a higher level of vascular oxidative stress in rats with genetically higher angiotensin I–converting enzyme levels. This effect is mediated through the angiotensin I receptor.

**Key Words:** angiotensin-converting enzyme ■ polymorphism ■ nitric oxide

Angiotensin I–converting enzyme (ACE) is a membrane-bound $Zn^{2+}$-metalloendopeptidase involved in the metabolism of angiotensin I to angiotensin II (Ang II) and in the hydrolysis of bradykinins.[1] ACE, expressed on the cell surface, plays a key role in blood pressure regulation and vascular remodeling.[2,3] In addition, an active soluble form of ACE, derived from endothelial cells, is present in plasma. Initially, Ang II was identified as a peptide that controlled systolic blood pressure (SBP) based on regulation of renal salt and water homeostasis, central nervous system mechanisms, and vascular smooth muscle cell (VSMC) tone.[4] Later, Ang II was also found to exert long-term effects on tissue structure, including cardiac hypertrophy, vascular remodeling, and renal fibrosis.[4]

Associations between several cardiovascular diseases and polymorphisms of the renin-angiotensin system (RAS) genes have been reported, in particular for the insertion/deletion polymorphism of the human ACE gene, which is defined by the presence (insertion [I]) or absence (deletion [D]) of a 287-bp insert in intron 16 of the gene. This insert harbors a sequence very similar to a silencer element,[5] which may explain why subjects with 1 or 2 D alleles have ≈25% and 50% higher ACE levels than subjects with the II genotype. These higher ACE levels are observed in plasma[6,7] and at tissue sites.[8,9]

Previous identification of a microsatellite marker in the rat ACE gene, specifically in the intron 13 on chromosome 10, has also allowed differentiation of ACE polymorphism alleles among different rat strains[10,11] and their association with different levels of plasma ACE[12] and neutral endopeptidase.[13,14] This genetically determined ACE expression in rats enhances the chronic hypertensive response after the induction of renovascular hypertension.[15] In addition, a relationship between circulating Ang II and the development of hypertension was also observed in this experimental model of genetically modulated hypertension.[15] Additionally, rats with this genotype and higher plasma ACE activity developed more myocardial fibrosis compared with those rats with low plasma ACE in response to isoproterenol.[16]

From the Department of Cardiovascular Diseases, Medical School, P. Universidad Católica de Chile (J.E.J., M.P.O., A.P., J.B., A.G.), and Departamento de Bioquímica and Biología Molecular, Facultad Ciencias Químicas y Farmacéuticas (J.B., A.G., I.G., S.L.), ICBM, Facultad de Medicina (S.L.) and Centro FONDAP Estudios Moleculares de la Célula (S.L.), Universidad de Chile, Santiago.

Correspondence to Dr Jorge Jalil, Departamento de Enfermedades Cardiovasculares, Escuela de Medicina, P. Universidad Católica de Chile, Lira 85, Piso 2, Santiago, Chile. E-mail jjalil@med.puc.cl or Dr Sergio Lavandero, Departamento Bioquímica and Biología Molecular, Facultad Ciencias Químicas y Farmacéuticas, Universidad de Chile, Olivos 1007, Santiago 664-0750, Chile. E-mail slavander@uchile.cl

Accumulating evidence has shown that Ang II increases vascular oxidative stress as well as vasoconstriction.[17–21] Activation of the RAS in different tissues and plasma[17,18,21] enhances the vascular production of reactive oxygen species (ROS), in part through the activation of membrane-bound NAD(P)H oxidases.[15–19] These enzymes are present in endothelial cells, VSMCs, fibroblasts, and phagocytic mononuclear cells.[17–21]

There are no studies assessing the relationship of genetically increased ACE expression and Ang II levels with NADPH oxidase in the vascular system. Hypothetically, increased ACE and Ang II should be associated to increased NADPH oxidase activity and vascular production of superoxide ($O_2^{\bullet -}$) and with decreased NO levels, a process that could be mediated by the angiotensin type 1 receptor. Accordingly, we investigated here the aortic NADPH oxidase activity and production of $O_2^{\bullet -}$ and the activity of the NO system in homozygous Lewis (LL; genetically low ACE) and homozygous Brown Norway (BB; genetically high ACE) rats.

## Methods

### Experimental Design

The investigation conformed with the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences; ISBN 0-309-05377-3; 1996), and it was approved by an institutional review committee. Normotensive strains of male rats (7 weeks of age), homozygous $F_2$ LL (n=25), and BB strains (n=36), with contrasting levels of plasma ACE, were used.[13,15,16] The $F_2$ homozygous strains were obtained after mating male $F_0$ BB with female LL inbreed strains obtained from Charles River (Wilmington, Mass). 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.[13,15,16]

SBP was measured by the tail-cuff method. Genomic DNA was purified and amplified by polymerase chain reaction (PCR).[13,15,16] Blood samples for Ang II and ACE measurements were rapidly collected after decapitation (nonanesthetized animals). Plasma was stored at −80°C. Aorta was rapidly excised, weighed, sectioned into slices, and stored at −80°C until determinations. In other experiments, aortic rings were used for vascular reactivity assay.

### ACE Activity

ACE was measured in plasma and aorta by spectrofluorimetry based on the hydrolysis of Z-phenyl-L-histidyl-L-leucine (Bachem), an analogous substrate for ACE.[13,15,16] Protein concentration was measured according to Bradford.[22]

### Plasma and Aorta Ang II Levels

Ang II was determined according to Admiraal et al.[23] Angiotensins were separated by reverse-phase high-performance liquid chromatography,[24] and concentrations of Ang II were measured by radioimmunoassay using an antibody kindly donated by Dr A.H J. Danser (Erasmus Universität, Rotterdam). Ang II recovery from tissue was 65%, and from plasma, 78%. The coefficients of variation for interassay and intra-assay variances were 13% and 6.5% for Ang II, respectively.[29]

### Measurement of $O_2^{\bullet -}$ Production

Vascular $O_2^{\bullet -}$ production was estimated with lucigenin-enhanced chemiluminescence.[19,25,26] Aortic segments were placed in chilled modified Krebs/HEPES buffer and homogenized on ice with a glass/glass tissue homogenizer for 2 minutes in 50 mmol/L PBS, which contained 0.01 mmol/L EDTA. The homogenate was centrifuged at 1000*g* for 10 minutes. The pellet was discarded, and the supernatant was stored on ice until use. After 5 minutes of dark adaptation, scintillation vials containing 2 mL Krebs–HEPES buffer with 50 μmol/L lucigenin were placed into a luminometer. Chemiluminescence values were obtained at 30-s intervals over 5 minutes. Lucigenin count was expressed as counts per second per gram of protein.

### NAD(P)H Oxidase Activity

Aorta was washed with ice-cold PBS and homogenized in cold lysis buffer (20 mmol/L $KH_2PO_4$, pH 7.0, 1 mmol/L EGTA, 10 μg/mL aprotinin, 0.5 μg/mL leupeptin, 0.7 μg/mL pepstatin, and 0.5 mmol/L PMSF). The homogenate was centrifuged at 1000*g* for 10 minutes at 4°C. The pellet was resuspended in a lysis buffer containing protease inhibitors and manually homogenized on ice. NADPH oxidase activity was measured by a luminescence assay in a 50 mmol/L phosphate buffer, pH 7.0, containing 1 mmol/L EGTA, 150 mmol/L sucrose, 5 μmol/L dark-adapted lucigenin as the electron acceptor, and 100 μmol/L NADPH as the substrate in a final volume of 900 μL. The reaction was started by the addition of 100 μL of homogenate, and luminescence measurements were obtained every 15 s for 5 minutes. Protein content was determined in an aliquot of the homogenate,[22] and the results were standardized to this measurement.

### Determination of Aortic NO Synthase mRNA Levels

Total RNA was isolated from the left ventricle (LV) as described previously.[16] RNA pellets were suspended in distilled water, and their concentrations were quantified by UV spectroscopy, assuming 40 μg/mL for 1 absorbance unit. RNA integrity was assessed from the intensity of the staining with ethidium bromide of 18 and 28S ribosomal RNA after agarose electrophoresis. The expressions of tissue endothelial NO synthase (NOS) and inducible NOS (iNOS) were evaluated by RT-PCR. For reverse transcription, 1 μg RNA was incubated with or without reverse transcriptase (Gibco-BRL) in a mixture containing random hexamers, deoxynucleotides, and RNAse inhibitor in reverse transcription buffer. For amplification of the resulting cDNA, 0.4 μmol/L endothelial NOS (eNOS)– or iNOS-specific primers, 0.2 mmol/L deoxynucleotides, 1.5 mmol/L $MgCl_2$, and 4 U *Taq* polymerase (Gibco-BRL) were added to 6 μL of each RNA sample in 50 μL. iNOS sequences of the sense and antisense primers were 5′-CACCTATGTAAGATCGCTTC-3′ and 5′-GCACAATCGCCATAATTATCC-3′, respectively,[27] yielding an amplification fragment of 227 bp. eNOS sequences of the sense and antisense primers were: 5′-CGCTACAACTTCGACTGGTGG-3′ and 5′-TATTTCCGGGATGTGGCCAT-3′, yielding an amplification fragment of 340 bp. GADPH sequences of the sense and antisense primers were: 5′-CCA TCA CCA TCT TCC AGG AG-3′ and 5′-CCT GCT TCA CCA CCT TCT TG-3′, yielding an amplification fragment of 299 bp. Amplification conditions for eNOS and iNOS PCRs were as follows: denaturation at 94°C for 1 minute, annealing at 62°C for 1.5 minutes, and elongation at 72°C for 1.5 minutes for 28 cycles, with a final elongation step at 72°C for 7 minutes. Amplification conditions for GADPH were: denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and elongation at 72°C for 1 minute for 25 cycles, with a final elongation step at 72°C for 10 minutes. After PCR, the amplification products were fractionated on a 1.5% (w/w) agarose gel and visualized by staining with ethidium bromide. Band intensities were quantified by computerized densitometry and normalized with respect to GADPH mRNA.

### NOS Activity

Aortas were homogenized on ice with a Polytron. NOS activity was determined by measuring the conversion of [$^3$H]-L-arginine to [$^3$H]-L-citrulline. Tissue homogenates were incubated in 20 mmol/L HEPES buffer, pH 7.5, containing 10 mmol/L L-arginine and [$^3$H]-L-arginine (3 μCi/mL), L-valine (60 mmol/L), NADPH (1 mmol/L), calmodulin (30 nmol/L), calcium (2 mmol/L), and tetrahydrobiopterin (5 mmol/L), and for 20 minutes at 37°C. Reaction was stopped by adding 1 mL of ice-cold HEPES buffer, pH 5.5, containing EGTA (2 mmol/L) and EDTA (2 mmol/L) and then applying to Dowex 50W ($Na^+$ form) columns, and the amount of [$^3$H]-L-citrulline eluted was quantified by

liquid scintillation counter. The activity of the $Ca^{2+}$-dependent NOS was determined from the difference between the [$^3$H]-L-citrulline produced from samples containing 2 mmol/L calcium and samples without calcium and with EGTA (2 mmol/L); the activity of the iNOS was determined from the difference between samples containing 2 mmol/L EGTA and samples without NADPH. Protein determination was made using dye-binding assay (Bio-Rad) with BSA as a standard.

## NOS Protein Levels

Aorta lysates containing 10 mg protein were denatured, and an equal amount of protein was loaded on 7.5% SDS-PAGE and then transferred to nitrocellulose membranes. The membrane was blocked with 1% BSA in Tris-buffer solution (TBS), pH 8.0, containing 0.1% Tween-20 for 2 hours at room temperature and incubated overnight at 4°C with mouse monoclonal anti-iNOS or anti-eNOS antibody (1:2000 dilution; Transduction Laboratories) in TBS containing 0.1% Tween-20. The membrane was washed and finally incubated with a 1:1000 dilution of anti-mouse IgG conjugated to horseradish peroxidase for 1 hour at room temperature. After successive washes with TBS, the immunocomplexes were detected using an enhanced horseradish peroxidase/luminol chemiluminescence reaction and exposed to x-ray film for 3 to 5 minutes. The density of bands was quantified by densitometric scanning using Image software. We took the density of the band representing 1 mg eNOS- or iNOS-positive control purified from human endothelial cells and mouse macrophage lysates (Transduction Laboratories), respectively, as 100% to calculate the relative density of other bands on the same gel.

## Plasma Nitrite/Nitrate Concentrations

Plasma (100 $\mu$L) was mixed with 160 $\mu$L of 75 mmol/L $ZnSO_4$ and 140 $\mu$L of 80 mmol/L NaOH for 10 minutes and centrifuged at 6000*g* for 10 minutes to deproteinize the plasma. The supernatant (100 $\mu$L) was removed and incubated with 25 $\mu$L substrate buffer (NADPH, 0.6 mmol/L; flavine adenine dinucleotide, 5 mmol/L; nitrate reductase 2 U/mL, pH 7.6, at 37°C for 1 hour to convert $NO_3$ to $NO_2$. The total $NO_2$ assay was determined according to Misko et al.[28]

## Vascular Reactivity Assays

After euthanization, thoracic aortas were removed, freed from periadventitia, and cut in fragments, each 5 mm in length. Aortic rings were mounted between horizontal stainless steel wires in a 10-mL organ bath containing Krebs–bicarbonate buffer, pH 7.4 (composition, in mmol/L: 119.0 NaCl, 25.1 $NaHCO_3$, 10.1 glucose, 4.8 KCl, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 1.2 $KH_2PO_4$), at 37°C, which was continuously gassed with 95% $O_2$-5% $CO_2$. The lower wire was stationary, and the upper wire was connected to a force-displacement transducer (HDW100A Biopac) for measurement of isometric tension. The output from the force transducer was recorded on Amplifier (MP100 Biopac). The aortic rings were stretched progressively to achieve a resting tension of 2.5*g*, which was determined by previous experiments to be the minimum tension facilitating the development and maintenance of maximal contractions to 70 mmol/L KCl under these experimental conditions. Vessels were maintained in resting tension for ≈30 minutes. After stabilization at resting tension, the buffer was changed and the vessels were constricted with 0.1 $\mu$mol/L norepinephrine to achieve 50% of the maximal contraction. Relaxations to acetylcholine ($10^{-9}$ to $10^{-4}$ mol/L) were studied in rings precontracted to norepinephrine in each individual ring. The vessel integrity was tested by checking the vasodilatory response to sodium nitroprusside ($10^{-9}$ to $10^{-4}$ mol/L).

## Statistical Analysis

Results are presented as mean±SEM. Unpaired *t* test, 1-factor ANOVA followed by Student-Newman–Keuls test, and linear correlation tests were used. A *P* value ≤0.05 was considered statistically significant.

# Results

Similar morphometric and hemodynamic parameters of the rats are shown in Table 1.

**TABLE 1. General Characteristics, ACE Activity, and Ang II Levels in Rats With Different ACE Polymorphism**

| General Characteristics | LL (n=13) | BB (n=15) | BB+C (n=6) |
|---|---|---|---|
| Body weight (g) | 229±9 | 204±8 | 116±3* |
| RLVM (mg · 100/g) | 233±6 | 229±10 | 293±7 |
| SBP (mm Hg) | 99±3 | 101±2 | 105±4 |
| ACE and Ang II Levels | | | |
| Plasma ACE activity (U/L) | 113±15 | 229±12† | 274±40† |
| Plasma Ang II (pg/mL) | 238±31 | 422±21† | ND |
| Aorta ACE (U/mg prot) | 67±3 | 122±6† | ND |
| Aorta Ang II (pg/g) | 16±4 | 15±4 | ND |

Mean±SEM.

*$P$<0.05 vs BB; †$P$<0.05 vs LL.

BB+C indicates BB rats treated with candesartan; RLVM, relative LV mass; ND, non determined.

## ACE Activity and Ang II Levels

Plasma and aortic ACE activities and plasma Ang II levels were higher by 100% in the BB rats than in the LL rats (Table 1; $P$<0.05) whereas no differences were observed in aortic Ang II content (Table 1).

## Aortic $O_2^{\bullet-}$ Production and NADPH Oxidase Activity and Effect of Blocking the Ang II Type 1 Receptor

As shown in Figure 1, $O_2^{\bullet-}$ production was significantly increased in the aorta from BB rats by 300% compared with LL rats ($P$<0.01). To verify the specificity of the lucigenin assay for superoxide production, the effect of the enzymatic excavenger superoxide dismutase (200 U) was tested. The addition of superoxide dismutase significantly inhibited $O_2^{\bullet-}$ production in aorta (data not shown).

To investigate the role of the flavin-containing enzymes NADPH oxidase and NOS in the $O_2^{\bullet-}$ production, aortic rings were treated with apocynin (2.5 mmol/L) as well as with diphenylene iodonum (10 $\mu$mol/L). The addition of apocynin and diphenylene iodonum significantly inhibited $O_2^{\bullet-}$ production in aorta from LL and BB rats (data not shown).

Aortic NADPH oxidase activity was increased by 260% in BB rats compared with LL rats (P<0.01; Figure 2). A significant correlation was observed between aortic ACE activity and NADPH oxidase activity ($r$=0.65; $P$<0.01).

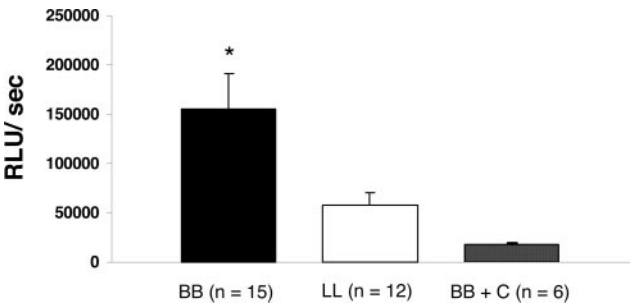


**Figure 1.** Superoxide production in the aorta from rats with different ACE expression. Mean±SEM. *$P$<0.05 vs the other groups (after a significant ANOVA). BB+C indicates BB rats treated with candesartan (10 mg/kg per day for 10 days); RLU, relative light units.

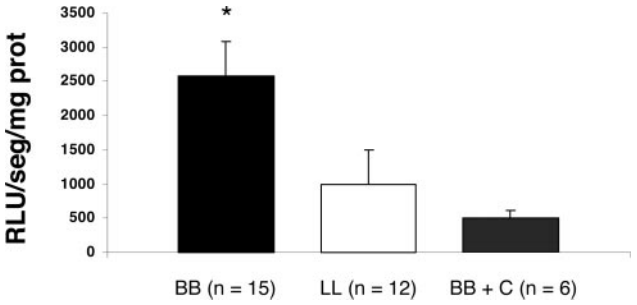


**Figure 2.** NADPH oxidase activity in the aorta from rats with different ACE expression. Mean±SEM. *$P<0.05$ vs the other groups (after a significant ANOVA). BB+C indicates BB rats treated with candesartan (10 mg/kg per day for 10 days); RLU, relative light units.

To evaluate if higher $O_2^{\bullet -}$ production and NADPH oxidase activity are dependent on the stimulation of the Ang II type 1 ($AT_1$) receptor, a group of homozygous BB rats (n=6) was treated with 10 mg/kg per day candesartan (Laboratorio Saval) by gavage for 7 days and killed the next day. In this group, the levels of aortic $O_2^{\bullet -}$ and NADPH oxidase activity were not increased and were similar to those in the homozygous LL rats (Figures 1 and 2, respectively). No significant change in SBP was observed with candesartan in these normotensive rats (107±3 mm Hg versus 105±4 mm Hg).

### NO System

eNOS activity in the aorta from BB rats was significantly lower than in LL rats (Table 2). However, iNOS activity in the aorta, eNOS and iNOS mRNA and their protein levels as well as serum levels of $NO_3^-/NO_2^-$ were similar in both genotypes.

### Aortic Relaxation in Response to Acetylcholine

The contraction response of the aortic rings to norepinephrine in LL and BB rats was similar. Relaxation in aortic rings was not different in response to $10^{-9}$ to $10^{-4}$ M acetylcholine between LL and BB rats. Maximal relaxation, in response to $10^{-4}$ M acetylcholine, was 79±5% in LL and 74±7% in BB rats (NS), suggesting that baseline aortic reactivity is similar in both groups.

## Discussion

The RAS is compartmented between the circulating blood and pericellular spaces. In target cells, the interaction of Ang II with the $AT_1$ receptor triggers different effects, including an immediate functional calcium-dependent response, secondary hypertrophy, and a late proinflammatory and procoagulant responses. These late pathological effects are mediated by NADPH oxidase–generated ROS.[29] Some of these effects seem to be mediated by the $AT_1$b receptor.[30] As shown in Table 1, although aortic ACE activity and plasma Ang II levels were significantly higher in BB rats than in LL rats, aortic Ang II levels were similar in the 2 strains. This lack of parallelism between plasma and aortic Ang II levels has not been assessed previously.[15,16] In this experimental model, aortic and lung ACE enzymatic activities are 2-fold higher in BB than in LL rats.[13] On the other side, in the LV from BB rats, with significantly higher ACE than LL rats, no differences were observed in LV Ang II concentrations between both genotypes.[15] In genetically engineered mice carrying an inactivation of the ACE gene, Alexiou et al have recently shown that whereas plasma ACE was 70% lower in $ACE^{-/-}$ than in $ACE^{+/+}$ mice (with 2 ACE gene copies), blood Ang II levels were reduced in $ACE^{-/-}$ mice. Ang II levels were significantly higher in the kidney, heart, lung, and adrenals in the $ACE^{+/+}$ mice than in the $ACE^{-/-}$ mice, whereas angiotensin I and Ang II levels in the brain and aorta were below the detection limit, despite the pooling of tissues to increase the sensitivity of the assay.[31] We believe that increased aortic ACE activity and aortic NADPH oxidase activity, as well as $O_2^{\bullet -}$ production in our BB rats, plus the reversal of the 2 latter effects using the $AT_1$ receptor antagonist candesartan, could reflect functionally higher aortic Ang II levels because of genetically higher ACE levels in the BB rats that are under our current detection methods. Another mechanism for increased aortic NADPH oxidase activity and $O_2^{\bullet -}$ production in our BB rats and its reversal by candesartan are the higher levels of plasma Ang II levels acting on the aortic $AT_1$ receptor in rats with the BB genotype.

Ang II induces its pleiotropic vascular effects through NADPH-driven generation of ROS. They are key intracellular and intercellular messengers to modulate many signaling effectors, such as phosphatases, protein kinases, transcription factors, and ion channels. Induction of these signaling pathways leads to VSMC growth and migration, regulation of endothelial function, expression of proinflammatory mediators, and modification of extracellular matrix. In addition, ROS increase intracellular free $Ca^{2+}$ concentration, a major determinant of vascular reactivity. ROS also influence signaling molecules by altering the intracellular redox state and by oxidative modification of proteins. In physiological conditions, these events play an important role in maintaining vascular function and integrity. Under pathological conditions, ROS contribute to vascular dysfunction and remodeling through oxidative damage.[32] Ang II–stimulated endothelial NADPH oxidase activity is regulated through phosphorylation of p47/phox.[33] After administration of Ang II to rats for 7 days, the activity and the expression of NADPH oxidase was increased by a protein kinase C–dependent mechanism. It is reasonable to hypothesize that NADPH oxidase–induced $O_2^{\bullet -}$ production might trigger NOS uncoupling if exposure were long enough (these animals were only 7 weeks of age),

**TABLE 2. NO System Indexes in Aorta From Rats With Different ACE Polymorphism**

| NO System Indexes | LL (n=13) | BB (n=15) |
|---|---|---|
| eNOS activity (U/g protein) | 1.7±0.2 | 1.0±0.1* |
| iNOS activity (U/g protein) | 0.1±0.1 | 0.6±0.5 |
| eNOS mRNA levels | 0.5±0.2 | 0.5±0.1 |
| iNOS mRNA levels | 0.1±0.1 | 0.4±0.2 |
| eNOS protein levels | 1.1±0.2 | 1.0±0.1 |
| iNOS protein levels | 0.3±0.2 | 0.8±0.2 |
| Plasma NO (mmol/L) | 11.1±0.5 | 13.5±1.3 |

Data are mean±SEM (n=5–7 per group).
* $P<0.03$ vs LL.

which could lead to impaired NO/cGMP signaling and endothelial dysfunction in this animal model.[21]

Increased oxidative stress induced by Ang II is linked to phospholipase $A_2$ activation[34] associated to the membrane NADPH that produces $O_2^{\bullet -}$.[35] Vascular growth, contraction, and generation of $O_2^{\bullet -}$ in response to Ang II may be also determined in part by activation of phospholipase D.[36] In human arteries from normotensive subjects as well as from patients with essential hypertension, enhanced oxidative stress and augmented growth-promoting actions of Ang II are associated with increased activation of phospholipase D–dependent pathways that may contribute to vascular remodeling.[37,38] The increased vascular activity of NAD(P)H oxidase enhances the production of ROS by several pathways, including the increased activation of xanthine oxidase, the auto-oxidation of NADH, and the inactivation of superoxide dismutase.

Consequences of these genetically determined phenotypes might be the contribution to the development of atherosclerotic lesions in the long term. Monocyte extravasation into the vessel wall has been shown to be a critical step in the development of atherosclerosis. On activation, monocytes produce a burst of $O_2^{\bullet -}$ because of activation of the NADPH oxidase enzyme complex. Monocyte-derived $O_2^{\bullet -}$ contributes to oxidant stress in inflammatory sites, is required for monocyte-mediated LDL oxidation, and alters basic cell functions such as adhesion and proliferation.[39]

Generation of $O_2^{\bullet -}$ mainly dependent on NADPH oxidase is abnormally enhanced in stimulated mononuclear cells from hypertensive patients. This alteration could be involved in the diminished NO production observed in these patients. Stimulation of $O_2^{\bullet -}$ production by Ang II and endothelin-1 was higher in cells from hypertensives than in cells from normotensives.[40]

In our experiments, neither differences in NADPH vascular activity nor in the production of $O_2^{\bullet -}$ contributed to different blood pressure or to aortic relaxation in response to acetylcholine, which might be explained by similar components in the NO system. However, we observed higher levels of experimental hypertension in BB rats (Goldblatt model[15]).

## Perspectives

Because this is a genetic model of increased ACE and Ang II,[15] similar to humans with the D allele,[41] it is possible that in humans with this condition, baseline or induced vascular $O_2^{\bullet -}$ production and NADPH oxidase activity are also enhanced. This suggests the presence of a higher level of vascular oxidative stress in people with the D allele, which might explain why they develop a higher incidence of vascular diseases such as restenosis after coronary angioplasty[42,43] or the higher risk of hypertension that is observed in men.[44,45] In addition, this phenotype depends on the $AT_1$ receptor stimulation, which can be blocked.

In conclusion, the main finding of this study was that aortic $O_2^{\bullet -}$ production and aortic NADPH oxidase activity are enhanced in rats with genetically higher ACE activity, which is inhibited by an angiotensin I receptor blocker.

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