Angiotensin-Converting Enzyme 2 (ACE2) and ACE Activities Display Tissue-Specific Sensitivity to Undernutrition-Programmed Hypertension in the Adult Rat

Guillaume Rivière, Annie Michaud, Christophe Breton, Gilles VanCamp, Christine Laborie, Mihaela Enache, Jean Lesage, Sylvie Deloof, Pierre Corvol, Didier Vieau

Abstract—Human epidemiological studies have shown that low birth weight is associated with hypertension in adulthood. Rodent models of intrauterine growth retardation (IUGR) support these findings because offspring from undernourished dams develop hypertension. Angiotensin-converting enzyme 2 (ACE2) is a newly described renin-angiotensin system (RAS) component that competes with ACE for angiotensin peptide hydrolysis and therefore may modulate blood pressure. However, ACE2 potential participation in hypertension programming remains unknown, although RAS alterations were reported in IUGR models. Hence, we first investigated the tissue distribution of ACE2 and ACE in the rat and then whether hypertension programming differentially affects both enzymes. Using multiplex RT-PCR and in situ hybridization, we show that ACE2 mRNA is widely expressed and coregionalized with ACE. Moreover, tissues involved in blood pressure homeostasis (lung, heart, and kidney) express high levels of both enzymes. Enzymatic assays reveal that ACE2 and ACE are coactive in these tissues. Adult (4-month-old) offspring from 70% food-restricted dams throughout gestation (FR30 rats) present mild hypertension, impaired renal morphology, as well as elevated plasma angiotensin II and aldosterone, suggesting alterations of the systemic RAS. In FR30 rats, we show that ACE2 and ACE activities are increased only in the lung, whereas their mRNA expression is not significantly altered, showing that the enzymes display tissue-specific sensitivity to programming. Our results indicate that ACE2 and ACE are coexpressed in numerous rat tissues and that their increased activity in the lung of FR30 rats may participate in hypertension programming. (Hypertension. 2005;46:1169-1174.)

Key Words: angiotensin-converting enzyme • renin-angiotensin system • angiotensin II • rats • lung • kidney

Angiotensin-converting enzyme 2 (ACE2), an ACE homologue, has been characeterized recently in humans and in mice. Like ACE, ACE2 is a zinc-dependent peptidase of the M2-metallopeptidase family that is sensitive to chloride ion concentration. ACE2 is an essential regulator of heart function. However, ACE2 is not inhibited by the classical ACE inhibitors captopril and lisinopril, which are used as antihypertensive drugs. Within the RAS, ACE2 competes with ACE because it is capable of hydrolyzing the inactive decapptide angiotensin I (Ang I) into the nonapeptide Ang(1–9), thus decreasing the amount of Ang I available for pressor Ang II generation by ACE. To the same extent, ACE2 degrades the vasoconstrictor Ang II into vasodilator Ang(1–7), which may also be produced from Ang(1–9) hydrolysis by ACE. Thus, in contrast to ACE, ACE2 is hypothesized to participate in blood pressure (BP) decrease in mammals. It has been shown that different components of the RAS undergo a progressive increase during pregnancy. Pregnant Sprague-Dawley rats showed a 2-fold increase in kidney ACE2 protein as well as a 1.5-fold increase in kidney and urinary concentrations of its processed peptide Ang(1–7), suggesting a potential role for maternal ACE2 during fetal development. In humans, ACE2 was found at various levels in 72 tissues that also express ACE mRNA. Because ACE2 has also been demonstrated to be a functional receptor for SARS coronavirus, its tissue distribution seems of great importance and appears to be species specific. However, it remains, to our knowledge, not well characterized in the rat.

Epidemiological studies in humans have revealed that intrauterine growth retardation (IUGR) consecutive to adverse environment during fetal life is associated with the development of hypertension in adulthood. Different rodent models of IUGR-induced hypertension have been developed. Dexamethasone injections to pregnant dams lead to elevated BP in adult offspring. Similar observations arose from low-protein or Fe-restricted fed dams during pregnancy. The mechanisms

Received May 4, 2005; first decision May 26, 2005; revision accepted August 9, 2005.
The first 2 authors equally contributed to this work.
Correspondence to Professor Didier Vieau, UPRES-EA 2701, Laboratoire de Neuroendocrinologie du Développement, SN4, Université des Sciences et Technologies de Lille, 59655 Villeneuve d’Ascq Cedex, France. E-mail didier.vieau@univ-lille1.fr
© 2005 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org

DOI: 10.1161/01.HYP.0000185148.27901.fe
involved in raised BP are not fully understood, although elements of the RAS have been demonstrated to be altered in several rodent models of programmed hypertension. In the Fe-restricted model, plasma ACE concentration is increased, which may elevate vasconstrictor plasma Ang II levels. Conversely, the early use of the ACE inhibitor captopril or angiotensin type 1 receptor antagonist losartan restores a normal systolic BP in adults. Nevertheless, perturbations of the RAS differ according to the programming paradigm, but ACE2 and ACE have never been studied in the maternal undernutrition model, despite the presence of hypertension in their offspring. We hypothesized that ACE2 and ACE may contribute to programmed hypertension. However, tissue ACE2 activity as well as ACE2/ACE mRNA balance have never been investigated to date in programming models.

Therefore, we first investigated the tissue distribution of ACE2 mRNA in rat tissues and found that ACE2 and ACE are expressed in tissues known to be involved in BP homeostasis. We show that ACE2 and ACE are present in heart, lungs, and kidneys and exhibit tissue-specific activity patterns. Then we studied ACE2 and ACE in adult offspring from 70% food-restricted dams throughout pregnancy (FR30 rats) because BP was found to be increased in this model of maternal undernutrition. Plasma levels of the RAS effectors (Ang II and aldosterone) were monitored as well as plasma and kidney renin concentrations. Kidney morphology as well as functional parameters were also explored. ACE2- and ACE-specific activities and expression were monitored in the lungs, heart, and kidneys. Our results show that ACE2 and ACE activities are increased in the same proportions in the lungs of FR30 rats, which may participate in hypertension programming.

Methods
The expanded Methods section is available online at http://www.hypertensionaha.org.

Animals, BP Measurements, and Tissue Collection
Wistar-Kyoto rats (250 to 280 g; Charles River; L’Arbresle, France) were housed in laboratory conditions with free access to food and tap water. For FR30 rats, day 1 pregnant dams were randomized into 2 groups. Control dams had a normal calorific intake, and food-restricted dams received a diet reduced by 70% throughout pregnancy, respectively. At birth, litter size was equilibrated (n=8), and all rats were fed ad libitum. Adult (4-month-old) male offspring from normal-fed and 70% restricted-fed females constituted the control group and the FR30 group, respectively. BP was measured by the tail-cuff plethysmography method with a Letica LE5001 system. Organs were dissected on ice, weighed, snap-frozen in liquid nitrogen, and stored at –80°C.

Reverse Transcription
Total RNA was isolated from tissues by the guanidium-thiocyanate-phenol-chloroform method. RNAs were treated with DNAseI RNAse–free and reverse transcribed using dT-adapter with Murine-Moloney Leukemia Virus reverse transcriptase (Invitrogen) according to manufacturer instructions.

ACE2 and ACE mRNA Localization and Expression

Semi quantitative Multiplex RT-PCR
The presence of ACE2 and ACE mRNAs was investigated by semi-quantitative multiplex RT-PCR. This method has been described previously and validated. β-Actin was used as an internal standard. ACE2 and ACE mRNA distribution analysis was performed on 4-month-old control animals, embryonic day 14 (E14) pups (fetal brain), and dams (placenta, uterus, and mammary gland). Products were resolved by agarose gel electrophoresis, stained with ethidium bromide, and analyzed with a Bio-Rad GS-700 densitometer using the Multi-Analyst software (version 1.1; Bio-Rad Laboratories).

ACE2 In Situ Hybridization
The rat 644-bp ACE2 riboprobe was obtained from heart cDNA by PCR and subcloned into pGEM-T easy (Promega). Control animal tissue sections (12 μm) were hybridized as described previously.

Programming Effect on ACE2 and ACE Expression and on ACE/ACE2 mRNA Ratio
ACE2 and ACE mRNA expression was monitored in tissues of control and FR30 rats by semiquantitative multiplex RT-PCR as described above. Direct ACE/ACE2 mRNA expression ratios in tissues were determined as well. PCR products were analyzed as described above.

Kidney Morphology
The number of nephrons was estimated per arbitrary surface unit, and the glomerular surface/cortex surface ratio was determined with a computer-assisted image analysis system using the Multi-Analyst software version 1.1.

Plasma and Urine Na+ and K+ Concentrations
Sodium and potassium concentrations were measured using an ion-selective electrode and inductively coupled plasma atomic emission spectrometry as described previously.

Steroids Radioimmunoassay
Plasma aldosterone and corticosterone concentrations were measured by radioimmunoassay as described previously.

Ang II Assay
Ang II was measured by enzyme immunoassay using a commercial rat Ang II enzyme immunoassay detection kit.

Renin Concentrations
Plasma and tissue renin concentrations were measured by enzymatic assay as described previously.

ACE Activities
Tissues were homogenized in ACE homogenization buffer and centrifuged. Assays were performed on 0.5 to 10 μL of tissue supernatant or plasma in a reaction volume of 250 μL.

ACE activity studies were performed with Hip-His-Leu (5 mmol/L) as a substrate, as described previously, and were resolved by reverse high-performance liquid chromatography. The specificity of the reaction was confirmed by incubation in the presence of a specific ACE inhibitor: perindoprilat (10 μmol/L).

ACE2 activity studies were performed with Ang II as a substrate in the presence of protease inhibitors (perindoprilat, amastatin, and bestatin; 10, 100, and 100 μmol/L, respectively). The specificity of the reaction was confirmed by incubation in the presence of a specific ACE2 inhibitor: DX600. After incubation, conversion of Ang II to Ang (1–7) was measured quantitatively by reverse-phase high-performance liquid chromatography.

Statistical Analysis
All values are given as mean±SEM. Statistical analysis was performed using impaired Student’s t test or Mann–Whitney rank sum test when required (P<0.05 was considered significant).

Results
ACE2 and ACE Coregionalization
Rat tissues express various levels of the ACE2 mRNA. ACE2 mRNA expression was marked in several tissues, such as heart,
lungs, intestine, kidney, and placenta, and appeared to be much lower in the hypothalamus, pituitary, cerebellum, stomach, pancreas, adrenals, and E14 fetal brain. Weak or no signal was detected in the hippocampus, skeletal muscle, liver, spleen, testis, uterus, and mammary glands (Figure 1). In contrast, ACE was more easily expressed in the tissues examined. In situ hybridization (ISH) was performed in the same tissues and gave similar qualitative results. In the heart, ACE2 mRNA was confined to the vascular endothelium (the aorta arch, the coronary arteries, and the pulmonary vein). The lung exhibited widespread labeling. In the kidney, labeling was mostly visible in the medulla, corresponding to the renal tubules. In the intestine, ACE2 mRNA expression is restricted to the epithelium. The embryonic side of the placenta is strongly labeled, whereas the maternal side is not. No ACE2 expression could be detected by ISH in the liver or the spleen, nor in the testis, although Leydig cells had been shown previously to express ACE2 (Figure 2).

In all ACE2 mRNA-expressing tissues, we also detected various levels of ACE mRNA, indicating that ACE2 is coregionalized with ACE (Figure 1).

### Tissue ACE2 and ACE Enzymatic Activities in 4-Month-Old Control Rats

In the lungs, ACE2 activity was moderate, whereas ACE activity appeared to be the highest. In the heart, ACE2 and ACE activities were moderate, whereas in the kidneys, the relative proportions of ACE2 and ACE activities were respectively moderate and strong (Table 1, control group).

### Maternal Plasma Corticosterone Levels and FR30 Body Weights

Undernourished dams had increased plasma corticosterone levels during pregnancy at E14 (12.3 ± 3.2 μg/dL, n = 6) versus 40.9 ± 2.8 μg/dL, n = 5; P < 0.001; control versus restricted dams), confirming that elevated maternal glucocorticoids may participate to hypertension programming. FR30 rats born at term presented a body weight reduction by 26% (6.64 ± 0.14 g [n = 26] versus 4.91 ± 0.15 g [n = 36]; P < 0.001; control versus FR30). However, FR30 animals underwent catch-up growth, and their body weights were not different from control rats at 4 months (454 ± 8 g [n = 17] versus 436 ± 11 g [n = 18]; control versus FR30).

### BP, Ang II, and Aldosterone Plasma Levels in 4-Month-Old Rats

Four-month-old FR30 rats developed mild hypertension (systolic BP 104 ± 2 mm Hg [n = 5] versus 125 ± 2 mm Hg [n = 6], P < 0.0001; and mean BP 66 ± 2 mm Hg [n = 5] versus 79 ± 2 mm Hg [n = 6], P < 0.005; control versus FR30) but did not present a cardiac hypertrophy (heart weight/total body weight ratio: 2.43 × 10^{-3} ± 3.83 × 10^{-3} [n = 6] versus 2.47 × 10^{-3} ± 2.610^{-5} [n = 7]; control versus FR30). Ang II and plasma aldosterone levels were increased by 1.8- and 2.4-fold, respectively, in FR30 rats (Ang II 41.3 ± 6.1 ng/mL [n = 6] versus 73.8 ± 12.7 pg/mL [n = 7], P < 0.05; and aldosterone 0.47 ± 0.008 ng/mL [n = 5] versus 1.13 ± 0.24 ng/mL [n = 6], P < 0.05; control versus FR30).

### Kidney Morphology and Plasma/Urine Na+ and K+ Concentrations in 4-Month-Old Rats

The ratio kidney weight to total body weight was not affected by prenatal undernutrition (data not shown), whereas the number of nephrons was reduced in FR30 rats (8.41 ± 0.06 [n = 5] versus 5.47 ± 0.49 [n = 5]; P < 0.001; control versus FR30). Glomerular surface/cortex surface ratio was significantly reduced as well (6.96 ± 0.21% [n = 5] versus 4.47 ± 0.55% [n = 5]; P = 0.01; control versus FR30), indicating that individual glomerular size did not compensate for the loss of nephrons. Na+ and K+ concentrations in plasma and urine remained unchanged in FR30 rats (Table 2).

### Renin Concentrations in 4-Month-Old Rats

Plasma and kidney renin concentrations were not significantly affected in FR30 rats (Table 3).

### Programming Effects on ACE2 and ACE in 4-Month-Old FR30 Rats

#### ACE2 and ACE Expression

ACE2 and ACE mRNA expression in lung, heart, and kidney of FR30 rats was not significantly different from the control group when compared with β-actin (Figure 3). The ACE/ACE2 mRNA expression ratio in the heart, kidneys, lungs, intestine,
adrenal glands, and hypothalamus was not different between control and FR30 animals (data not shown).

**Tissue and Plasma Enzymatic Activities**

In FR30 rats, ACE2 and ACE activities were significantly increased in the lung (by 2.0- and 1.5-fold, respectively; \( P<0.05 \)), whereas they were not significantly altered in the heart and kidneys (Table 1). Plasma ACE activity was not different between groups, and plasma ACE2 activity was not detected (data not shown).

**Discussion**

This study reports ACE2 mRNA distribution and enzymatic activity in rat tissues. ACE2 mRNA displays a wide distribution and is coregionalized with ACE in all tissues. Similar results have been obtained in humans, confirming the hypothesis of a complete local tissue-related RAS. However, ACE2 mRNA expression was different in the organs examined. The highest ACE2 mRNA expression levels were detected in the intestine epithelium. Like rat intestinal ACE, which is stimulated by high-protein-feeding, ACE2 may act as a final proteolytic enzyme in nutrient degradation. The other major ACE2-expressing tissue was the placenta, suggesting that ACE2 may be involved in mother/fetus interactions, which is interesting regarding a potential role of ACE2 in fetal programming and pregnancy. ACE2 and ACE mRNA expression was elevated in structures involved in fluid homeostasis and BP regulation (over the entire lung, the vascular endothelium of the heart, and the region of the renal tubules).

Because ACE2 and ACE were active in lung, heart, and kidney in vivo, this would favor the existence of a functional local RAS. Like mRNAs, enzymes display tissue-specific activity patterns. ACE2 activity was the highest in the kidney. Consistently, Li et al demonstrated a strong Ang(1–7) generation in this location. ACE2 activity was moderate in the lung, where ACE activity was the highest. This suggests that, in control as well as in FR30 rats, the lungs are the major site of Ang II production, an observation that is consistent with the data obtained from tissue–ACE knockout mice. Coregionalization

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Na(^+) mmol/L</th>
<th>K(^+) mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=7)</td>
<td>139.41±4.17</td>
<td>10.28±0.41</td>
</tr>
<tr>
<td>FR30 (n=7)</td>
<td>148.97±4.08</td>
<td>11.98±1.12</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>57.21±17.00</td>
<td>122.72±30.20</td>
</tr>
<tr>
<td>FR30 (n=6)</td>
<td>55.41±18.40</td>
<td>112.58±26.70</td>
</tr>
</tbody>
</table>

**Table 2. Plasma and Urine Na\(^+\) and K\(^+\) Concentrations in 4-Month-Old Rats**

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>PRC, ng AngI/mL/h</th>
<th>RRC, ( \mu )g AngI/mgprot/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>control (n=5)</td>
<td>0.43±0.05</td>
<td>136.5±14.1</td>
</tr>
<tr>
<td>FR30 (n=6)</td>
<td>0.48±0.08</td>
<td>145.3±11.1</td>
</tr>
</tbody>
</table>

**Table 3. Plasma Renin Concentration (PRC) and Renal Renin Concentration (RRC) in 4-Month-Old Control and FR30 Rats**
of ACE2 and ACE mRNAs and ACE2 and ACE tissue-specific activity patterns suggest that these 2 enzymes may compete for their substrates and thus locally modulate RAS autocrine/paracrine physiological actions.

The hypertension observed in FR30 rats supports the fetal programming paradigm. Their low birth weight reflects an important role because maintenance of maternal diet-induced hypertension in the rat is dependent on glucocorticoids. The hypertension observed in FR30 rats supports the fetal programming paradigm. Their low birth weight reflects an important role because maintenance of maternal diet-induced hypertension in the rat is dependent on glucocorticoids. The kidneys of FR30 rats had normal size but exhibited reduced number of nephrons, which was not compensated by an increase in glomerular individual size. FR30 rats might thus have a loss of filtration surface accompanied by an increased renal resistance to the afferent blood flow that may participate in hypertension. Perturbations of the developing kidney have already been described in programming in the low-protein model, consistent with our present findings. However, FR30 rats have normal plasma and urine sodium and potassium concentrations and normal plasma creatinine (data not shown), in agreement with previous studies, suggesting that they seem to exhibit a normal renal function.

The elevated plasma levels of Ang II and aldosterone observed in FR30 animals is likely to contribute to the hypertension in those rats, as shown in previous studies, and reflects perturbations of the RAS. However, some of its parameters were not modified by maternal undernutrition: liver angiotensigen and kidney renin expression (data not shown), plasma and kidney renin concentrations, and plasma ACE activity remained unchanged in FR30 rats. Because ACE2 and ACE were coregionalized and both active in tissues involved in volume regulation, we hypothesized that an altered balance in these competing enzymes would contribute to an overproduction of Ang II. Until now, only plasma and lung ACE concentrations have been demonstrated to be increased in programming models. Because membrane-bound ACE is particularly catalytically active in vivo when compared with secreted ACE, tissue rather than plasma ACE activities appears to be involved in plasma Ang II production, as suggested in genetically engineered mice. For this reason, ACE2 and ACE activities were measured in the heart, lungs, and kidneys and found to be increased (by 2.0- and 1.5-fold, respectively) in the lung in FR30 rats. This increase in activities is not accompanied by an increase in their mRNA levels. Accordingly, elevated cardiac ACE2 activity without elevated cardiac ACE2 mRNA has been reported recently in rats, suggesting that translational or post-translational mechanisms might regulate ACE2 activity.

Because both enzymes present a similar increase in activity, one may argue that the resulting Ang II content should remain poorly affected. Nevertheless, taking into account the specific in vitro affinities of ACE2 and ACE for Ang I (K_m = 6.9 versus 18 μmol/L and k_cat/K_m = 4.9.10^5 versus 2.35.10^6 M^-1·s^-1, respectively), an increase in ACE2 activity should be 100-fold greater than an increase in ACE activity to maintain basal Ang II levels. Thus, Ang I would be preferentially cleaved to Ang II by ACE rather than to Ang(1–9) by ACE2. Therefore, the increase in lung ACE activity may partly explain the elevation in circulating Ang II in FR30 rats, which may in turn elevate aldosterone levels. Alternatively, programming could upregulate other Ang II–forming pathways. Interestingly, neither ACE2 nor ACE seems to be affected by programming in heart and kidney in FR30 rats. These data indicate that organs may display different sensitivities regarding fetal programming.

Perspectives
Together, our results show that ACE2 and ACE are coregionalized in numerous rat tissues, which supports the hypothesis of complete, functional local RAS. ACE2 expression is elevated in the intestine, placenta, and tissues known to be important regulators of BP and actors of the systemic RAS. ACE2 and ACE activities are upregulated in the lungs of FR30 rats but not in the heart or kidney, indicating that hypertension programming may be tissue specific. Although the lung is clearly a primary target of programmed hypertension by maternal undernutrition, other structures, such as adipose tissue or the central nervous system, may also undergo alterations that may in turn have systemic consequences. In any case, these issues require further investigations to fully understand the mecha-
anisms underlying the prenatal programming of adult metabolic disorders.

Acknowledgments

This study was supported partly by grants from the Conseil Régional du Nord-Pas de Calais and the Institut National pour la Santé et la Recherche Médicale. We are grateful to Pr Jean-François Barthe, Valérie Montel, Anne Dickès-Copman, and Françoise Lefèvre for technical assistance. We thank Jean-Michel Danger and Dr Dawn Halliday for critical reading of this manuscript.

References

Angiotensin-Converting Enzyme 2 (ACE2) and ACE Activities Display Tissue-Specific Sensitivity to Undernutrition-Programmed Hypertension in the Adult Rat
Guillaume Rivière, Annie Michaud, Christophe Breton, Gilles VanCamp, Christine Laborie, Mihaela Enache, Jean Lesage, Sylvie Deloof, Pierre Corvol and Didier Vieau

Hypertension. 2005;46:1169-1174; originally published online October 3, 2005; doi: 10.1161/01.HYP.0000185148.27901.fe

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/5/1169

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2005/11/01/01.HYP.0000185148.27901.fe.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/