Positron-Emission Tomography Imaging of the Angiotensin II Subtype 1 Receptor in Swine Renal Artery Stenosis

Jinsong Xia, Esen Seckin, Yan Xiang, Melin Vranesic, William B. Mathews, Kelvin Hong, David A. Bluemke, Lilach O. Lerman, Zsolt Szabo

Abstract—The angiotensin II subtype 1 receptor (AT,1R) has been linked to the development and progression of renovascular hypertension. In this study we applied a pig model of renovascular hypertension to investigate the AT1R in vivo with positron-emission tomography (PET) and in vitro with quantitative autoradiography. AT,1R PET measurements were performed with the radioligand [11C]KR31173 in 11 control pigs and in 13 pigs with hemodynamically significant renal artery stenosis; 4 were treated with lisinopril for 2 weeks before PET imaging. The radioligand impulse response function was calculated by deconvolution analysis of the renal time-activity curves. Radioligand binding was quantified by the 80-minute retention of the impulse response function. Median values and interquartile ranges were used to illustrate group statistics. Radioligand retention was significantly increased (P = 0.044) in hypoperfused kidneys of untreated (0.225; range: 0.150 to 0.373) and lisinopril-treated (0.237; range: 0.224 to 0.272) animals compared with controls (0.142; range: 0.096 to 0.156). Increased binding of [11C]KR31173 documented by PET in vivo was confirmed by in vitro autoradiography. Both in vivo and in vitro binding measurements showed that the effect of renal artery stenosis on the AT1R was not abolished by lisinopril treatment. These studies provide insight into kidney biology as the first in vivo/in vitro experimental evidence about AT1R regulation in response to reduced perfusion of the kidney. The findings support the concept of introducing AT1R PET as a diagnostic biomarker of renovascular disease. (Hypertension. 2008;51[part 2]:466-473.)

Key Words: positron-emission tomography ■ angiotensin AT1 receptor ■ swine ■ animal models ■ renovascular hypertension

Renovascular hypertension is the most common type of secondary hypertension that occurs in 2 to 4 million people in the United States. The renin-angiotensin system and its component, the angiotensin II subtype 1 receptor (AT1R), have been tightly linked to the development and progression of renovascular hypertension (RVH), but experimental mechanistic evidence for regulation of the AT1R has not been established in vivo. Antagonists against the angiotensin-converting enzyme (ACE; ACE inhibitors) or the AT1R (angiotensin receptor blockers) continue to play a key role in the therapy of renal hypertension and other kidney diseases.1 Both drugs represent a “double-edged sword,” because they may deactivate regulatory effects of the renin-angiotensin system and the AT1R and trigger irreversible renal damage.2 The complexity of the management of RVH raises a pressing need for an in vivo imaging technique that cannot only demonstrate reduced blood flow to the organ but also detect and monitor renal ischemia at the molecular level.3–6

Magnetic resonance (MR) angiography (MRA) and computed tomography angiography are oriented toward depicting anatomy rather than tissue injury.7,8 Radionuclide captopril renography has not been widely accepted9 because of its limited accuracy in the presence of bilateral disease10 or therapy with ACE inhibitors.11 Inhibiting the activity of the AT1R with angiotensin receptor blockers or ACE inhibitors, on the other hand, is widely adapted by clinicians to protect the kidney from renal injury.12–16

Probing molecular changes is expected to assist diagnosis and support prediction and evaluation of treatment success. We have identified the AT1R as a significant molecular imaging target because of its intricate involvement in the many aspects of renal physiology and pathology,17–19 particularly in acute, subacute, and chronic complications of renal ischemia.20,21 The AT1R is a key injury response protein because its continuing activation leads to stimulation of the extracellular matrix,22 collagen deposition, glomerular remodeling,23 inflammation,24 apoptosis,25 generation of oxygen species, and cell cycle arrest.26

To this end, our group has synthesized several radioligands and tested them in various animal models for positron-emission tomography (PET) imaging of the renal AT1R.27–31

Received October 9, 2007; first decision October 31, 2007; revision accepted December 4, 2007.
From the Departments of Radiology (J.X., E.S., M.V., W.B.M., K.H., D.A.B., Z.S.) and Physiology (Y.X.), Johns Hopkins Medical Institutions, Baltimore, Md; Department of Medicine (L.O.L.), Mayo Clinic College of Medicine, Rochester, Minn.
Correspondence to Zsolt Szabo, Johns Hopkins Outpatient Center, Room JHOC 3233, 601 N Caroline St, Baltimore, MD 21287-0817. E-mail zszabo@jhmi.edu
© 2008 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.107.102715
One particular radioligand, $[^{11}C]$KR31173, has shown excellent image quality in multiple animal species including nonhuman primates. Our previous studies have established PET imaging for studying the regulation of the AT$_1$R and its response to dietary sodium manipulation and varying 17-$\beta$-estradiol levels under in vivo conditions. The present experiments were designed to study how the renal AT$_1$R is regulated in the swine model of renal artery stenosis and whether the changes of AT$_1$R binding are affected by treatment with ACE inhibitors. Pigs were chosen because the anatomy and physiology of the pig kidney are well characterized, and it shows great similarities with humans. Cortical-medullary tissue distribution of the AT$_1$R is also comparable between pigs and humans.\textsuperscript{35}

Renal artery stenosis was induced by copper-coated wire coils inserted into one of the proximal pig renal arteries. The presence of a hemodynamically significant stenosis was established by $[^{15}O]$water PET and MRA. The in vivo accumulation and binding of $[^{11}C]$KR31173 was measured with PET to estimate the density of the AT$_1$R protein. The in vivo binding data were corroborated with in vitro quantitative autoradiography of the AT$_1$R.

### Methods

#### Synthesis of $[^{11}C]$KR31173

KR31173 (2-butyl-5-methoxy methyl-6-(1-oxopyridin-2-yl)-3-[[2-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-3H-imidazo[4,5-b]pyridine) was radiolabeled by coupling a tetrazole-protected hydroxy precursor with $[^{11}C]$methyl iodide and removing the protecting group by acid hydrolysis.\textsuperscript{32} The originally published procedure\textsuperscript{30} was modified by using anhydrous tetrahydrofuran as the reaction solvent.\textsuperscript{32} (The precursor was obtained from the Center for Biological Modulators/KRICT.) The average time of synthesis, purification, and formulation was 31 minutes. At the end of synthesis the nondecay corrected radiochemical yield was 5.3%, the radiochemical purity was >99%, and the specific activity was 260 GBq/μmol (7000 mCi/μmol).

#### Animal Model of RVH

Domestic pigs with an average age of 2.5 months and average weight of 25 kg were maintained on a regular diet. The study was approved by the Johns Hopkins Animal Care and Use Committee. Renal artery stenosis was induced by intravascular tissue-irritating copper coils\textsuperscript{36} in 21 animals. This technique results in ~70% stenosis of the renal artery within 2 to 3 weeks. Under general anesthesia and sterile conditions, a vascular surgical cutdown was performed at the common femoral artery in the right groin, and a 6F French vascular sheath (Cordis) was put in place. A 5F pigtail catheter (Cook, Inc) was advanced over a Bentsen guide wire (Cook, Inc) through the vascular sheath, and an abdominal angiogram was performed. The right or left renal artery was accessed using an SOS-3 catheter (AngioDynamics), and an SV-5 wire (Cordis Warren) was placed in the renal artery. Eleven animals without renal artery stenosis served as controls. A group of animals with renal artery stenosis also received treatment with the ACE inhibitor lisinopril, which was given orally mixed with food in a daily dose of 0.75 mg/kg from the day of surgery until the day of sacrifice.

#### Preparation for Imaging

Blood samples were collected before each PET study for determination of red blood cell counts, hemoglobin, hematocrit, electrolytes (Na, K, and Cl), blood urea nitrogen, and serum creatinine. The animals were fasted for 12 hours before MR and PET. An ear vein was cannulated for induction and maintenance of anesthesia. A second ear cannula was placed for injection of the radioligand or the MR contrast agent. Anesthesia was maintained with a mixture of 43.5 mL of ketamine and 6.5 mL of xylazine added to 1 L of normal saline. Heparin (50 000 U) was added to this solution to prevent thrombosis. The rate of infusion was set at 3 mL/kg per hour. However, under anesthesia, the animals were intubated for mechanical ventilation with room air. A vascular cutdown in 1 of the femoral arteries was performed to draw multiple blood samples during the PET study. Arterial blood pressure and heart rate were monitored using the same arterial access.

#### MRA

MRA was performed under general anesthesia with a 1.5-T General Electric CV/i MR scanner. A noncontrast 3D spoiled gradient-recalled echo sequence was obtained first in the coronal plane. Imaging covered the entire anterior-to-posterior dimensions of the kidneys. The most anterior slices were extended 1 to 2 cm anterior to the aorta. MR scan parameters included repetition time/echo time minimum, 256×224 (frequency×phase), field of view of 26 (adjusted for animal size), flip angle 30°, slice thickness of 2 mm, and in-line subtraction imaging. Zero-filling of k-space was performed in the slice direction, as well as in plane. After successful performance of the noncontrast MR scan, 0.2 mL/kg of gadodiamide was injected at 2 mL/s, followed by administration of a 15-cc saline bolus at the same rate. Fluoroscopic triggering of the sequence was performed on identification of the contrast bolus in the abdominal aorta. Four postcontrast image data sets were acquired at a 20-second temporal resolution. Acquisitions were obtained during suspension of ventilator respiration. Targeted maximum intensity projection images were obtained in the oblique coronal and axial planes for display of the renal arteries. Maximum intensity projection angiograms were processed and displayed on a Siemens Leonardo workstation. Segmentation algorithm was used for assessment of the percentage of stenosis of the renal artery.

#### PET

The kidneys were localized with an ultrasound device (Ultramark 4, Advanced Technology Laboratories), and the animals were positioned with both kidneys in the PET field of view. PET imaging was performed with a GE Advance PET scanner. A transmission scan was obtained with a pair of 370-MBq Germanium-68 pin sources. First, renal blood flow imaging was performed with PET after intravenous bolus injection of 740 MBq (20 mCi) of $[^{15}O]$water using an image sequence of 24 five-second frames followed by 6 ten-second frames. Subsequently, a dynamic PET study was performed with injection of 518±200 MBq (14.2±6 mCi) of $[^{11}C]$KR31173 at an average specific activity of 206 GBq/μmol (5580 mCi/μmol). Thus, specific activity was 80% of the specific activity at end of synthesis, which translates to a decay time of 6.5 minutes. The image sequence of the receptor PET study was: four 15-second scans, three 1-minute scans, three 2-minute scans, three 5-minute scans, three 10-minute scans, and two 20-minute scans. Two animals with renal artery stenosis, 1 untreated and 1 lisinopril treated, were used to investigate the degree of non-specific radioligand binding. After the baseline receptor PET study, these animals received 1 mg/kg of the potent AT$_1$R antagonist SK-1080 (2-butyl-5-methoxy methyl-6-(1-oxopyridin-2-yl)-3-[[2-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]-3H-imidazo[4,5-b]pyridine; Center for Biological Modulators/KRICT)\textsuperscript{37,38} injected slowly intravenously 30 minutes before the second receptor PET study.

#### Input Function

Carotid artery blood samples (5 mL) were collected at 6 time points (0, 5, 15, 30, 60, and 90 minutes after injection) to measure the unmetabolized tracer concentration in plasma by column-switch high-performance liquid chromatography.\textsuperscript{32,39} Smaller volumes (0.5 mL) of blood were collected from the carotid artery every 5 seconds during the first 2 minutes and then at increasing time intervals of 1 to 10 minutes until the end of the study (total: 15 mL). Exact times of blood sampling were recorded. Aliquots of 0.2 mL of plasma were obtained by centrifugation and were counted in a well-type counter.
PET Scan Processing

PET scans were reconstructed using ramp filtered back projection in a 128×128 matrix, with a transaxial×transaxial×axial voxel size of 2×2×4 mm. Image smoothing was applied only for display purposes. To obtain time-activity curves (TACs), regions of interest were marked around the renal cortex including activities ≤20% peak activity. The resulting TACs were corrected for isotope decay and injected dose and expressed in units of mCi/mL/mCi (Bq/mL/MBq). To account for variances in radioligand administration, metabolism, and recirculation, the TACs were processed by least-squares deconvolution analysis using the arterial input function, which resulted in a standardized TAC called the impulse response function.

In Vivo Binding Parameters

Using the individual kidney TACs obtained with [15O]water, an area under curve value was obtained from onset of activity to first peak. A perfusion ratio was calculated as the area under curve ratio between stenotic and contralateral kidneys (right-to-left ratio was calculated in control animals). Only animals with ≥10% reduction in the perfusion ratio were included in the stenosis group. Impulse response function peak values, fmax, were used to calculate the perfusion ratio from the [15O]water TACs. Radioligand binding was characterized by the 80-minute retention value (fret=f0 min/fmax) of the impulse response function.

Tissue Collection

On completion of the last PET study for each pig, animals were euthanized by an overdose of pentobarbital and both kidneys were quickly separated from surrounding connective and adipose tissue and rinsed in ice-cold sterile saline. Kidney sections were snap frozen in liquid nitrogen without separating cortex from medulla and were stored at −80°C until further study. Frozen tissue samples of the control and stenotic kidneys were sectioned in a cryostat at 80°C until used.

Autoradiography

Autoradiography was carried out in an assay medium containing 150 mmol/L of NaCl, 1 mmol/L of EDTA, 0.1 mmol/L of bacitracin, and 50 mmol/L of NaPO4 (pH 7.2). After a 30-minute preincubation period in the assay medium at 22°C to 24°C, 4 adjacent tissue sections were incubated for 2 hours with [3H]-[Sar1Ile8] Ang II (Ang II) in the presence or absence of subtype selective angiotensin receptor antagonists to obtain the following measurements: (1) total binding with no antagonist added; (2) AT1R binding with 10 μmol/L of SK-1080 added; (3) AT1R binding with 10 μmol/L of PD-123,319 added; and (4) nonspecific binding with 200 nM unlabeled Ang II added. The sections were then rinsed sequentially in water and assay medium without radioligand, rinsed again in water, and dried under a stream of cool air. Finally, the sections were exposed to x-ray film for 10 days. The latent images of [3H]-[Sar1Ile8] Ang II binding on the film were captured with a digital camera and quantified with Labworks Image Acquisition and Analysis Software (UVP Bioimaging Systems).

Statistical Analysis

To account for potential non-Gaussian distribution, each group was represented by its parameter median value and interquartile range, and the data were graphically displayed as box plots. Box plots were constructed to display the median, interquartile range, and 99% CI of each animal group. Blood pressure was expressed with the mean and SD.

The primary hypothesis was that AT1R binding (fmax) was increased in both untreated and lisinopril-treated kidneys with renal artery stenosis. This hypothesis was tested by Kruskal-Wallis ANOVA of the binding parameters fmax and Bmax. Because this multiparametric test did not differentiate between untreated and lisinopril-treated animals with renal artery stenosis, differences between those 2 groups were tested with the Mann-Whitney U test.

One explorative hypothesis was that [11C]KR31173 could be used for calculation of the perfusion ratio. For this purpose, the perfusion ratios obtained with [11C]KR31173 were compared with the perfusion ratios obtained with [15O]water as the reference using Pearson’s correlation. Secondly, the relationship between the in vitro and in vivo binding parameters Bmax and fmax was investigated by Spearman’s correlation analysis.

Results

Clinical chemistries, including blood urea nitrogen and creatinine, were within reference ranges in all 3 of the animal groups (Table 1). Renal artery stenosis induction was successful in 13 pigs (Figure 1, including 9 untreated and 4 lisinopril-treated (ACE inhibitor) animals. In these kidneys, MRA demonstrated renal artery narrowing and decreased contrast accumulation; [15O]water PET showed >10% reduction of the perfusion ratio. Coils were placed in additional 8 animals that were excluded from the study: 5 because of a

Table. Clinical Chemistry Values in Control, Untreated, and Lisinopril-Treated Pigs With Renal Artery Stenosis

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Creatinine, mg/dL</th>
<th>Sodium, mEq/L</th>
<th>Potassium, mEq/L</th>
<th>Chloride, mEq/L</th>
<th>BUN, mg/dL</th>
<th>RBC, 10⁶/µL</th>
<th>WBC, 10⁶/µL</th>
<th>HGB, g/dL</th>
<th>HCT, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.2 (1.1 to 1.3)</td>
<td>141 (140 to 142)</td>
<td>4.7 (4.5 to 5.3)</td>
<td>104 (101 to 105)</td>
<td>8.0 (7.0 to 9.5)</td>
<td>6.5 (6.1 to 6.7)</td>
<td>13.4 (12.5 to 14.0)</td>
<td>10.6 (9.6 to 11.8)</td>
<td>46 (43 to 48)</td>
</tr>
<tr>
<td>Stenosis</td>
<td>1.3 (1.2 to 1.5)</td>
<td>140 (139 to 142)</td>
<td>4.5 (4.2 to 5.1)</td>
<td>101 (96 to 102)</td>
<td>11.0 (9.3 to 12.8)</td>
<td>7.1 (6.3 to 8.2)</td>
<td>13.5 (10.3 to 15.0)</td>
<td>11.9 (10.5 to 14.0)</td>
<td>51 (46 to 61)</td>
</tr>
<tr>
<td>Stenosis + lisinopril</td>
<td>1.3 (1.2 to 1.6)</td>
<td>140 (137 to 142)</td>
<td>4.3 (3.9 to 5.1)</td>
<td>99 (96 to 105)</td>
<td>14.0 (9.0 to 22.5)</td>
<td>6.5 (5.5 to 7.5)</td>
<td>13.1 (10.2 to 13.3)</td>
<td>10.7 (9.1 to 12.5)</td>
<td>47 (40 to 54)</td>
</tr>
</tbody>
</table>

Data are median and interquartile range. Control indicates control pigs; stenosis, untreated; stenosis + lisinopril, lisinopril treated; BUN, blood urea nitrogen; RBC, red blood cell count; WBC, white blood cell count; HGB, hemoglobin; HCT, hematocrit. Parameter differences among the 3 animal groups are not statistically significant (P>0.05).

Figure 1. MRA images obtained in a pig with stenosis of the right renal artery. The maximum intensity projection image (A) shows narrowing of the right renal artery (arrow). The coronal scan (B) confirms reduced contrast accumulation in the right kidney.
complete occlusion of the renal artery and 3 because of a hemodynamically insignificant stenosis of the renal artery determined by MRA.

Arterial blood pressure was increased in animals with renal artery stenosis: 153±16/106±11 mm Hg in untreated and 132±13/89±7 mm Hg in lisinopril-treated animals. Blood pressure was 120±18/87±11 mm Hg in the control group.

The following perfusion ratios were measured with [15O]water (Figure 2): control 1.047 (range: 0.928 to 1.134), stenosis untreated 0.466 (range: 0.326 to 0.660), and stenosis lisinopril treated 0.437 (range: 0.358 to 0.589). The correlation coefficient of the perfusion ratios obtained with [15O]water and [11C]KR31173 was $r=0.938$ (Figure 3).

Figure 4 shows the uptake (2 minutes) and retention (30 minutes) of [11C]KR31173. The 30-minute image was
chosen to illustrate radioligand retention because of the exceptional quality of the image at this time point. In control kidneys, uptake and retention were symmetrical (Figure 4A and 4B). In stenotic kidneys, radioligand uptake was decreased (Figure 4C, arrow) and radioligand binding was increased (Figure 4D).

The TACs obtained with [11C]KR31173 showed that radioligand concentration in the kidneys peaked at 2 to 4 minutes postinjection. The tissue activity peak value (expressed in nCi/mL/mCi of injected dose, identical to Bq/mL/MBq) was decreased both in the untreated animals (569; range: 192 to 752) and in the lisinopril-treated animals (256; range: 204 to 391) compared with controls (852; range: 756 to 1063). In control kidneys (Figure 5A) and kidneys contralateral to stenosis (Figure 5B), activity declined first rapidly and then slowly to reach 20% of the peak 75 to 95 minutes postinjection. In stenotic kidneys, the TACs showed a reduced peak, a reduced rapid component, and an unchanged slow component (Figure 5B). Pretreatment with the potent AT1R inhibitor SK-1080 suppressed the TAC. The effect of SK-1080 on the slow binding component was similar in control kidneys (Figure 5A), stenotic kidneys, and contralateral kidneys (Figure 5B), and estimated specific binding (difference between pre–SK-1080 and post–SK-1080 scans) was 70% to 80%.

In vivo radioligand binding ($f_{ret}$; Figure 6), was increased ($P=0.044$) in the stenotic kidneys of both untreated (0.225; range: 0.150 to 0.373) and lisinopril-treated (0.237; range: 0.224 to 0.272) animals compared with controls (0.142; range: 0.096 to 0.156). The differences between the 2 subgroups of animals with renal artery stenosis were statistically not significant ($P=0.877$).

The autoradiographic images showed high in vitro AT1R binding in the medulla, as well as in a punctuated pattern in the cortex (Figure 7). The distribution of activity indicated predominantly tubular-epithelial binding in the medulla and glomerular binding in the cortex. Quantitative analysis of the images revealed significantly increased cortical AT1R binding (Figure 8A) in stenotic kidneys with the following $B_{\text{max}}$ values: control kidneys at 0.100 fmol/mg (range: 0.092 to 0.111 fmol/mg); stenosis kidneys untreated at 0.233 fmol/mg (range: 0.190 to 0.378 fmol/mg); and lisinopril-treated stenosis kidneys at 0.314 fmol/mg (range: 0.294 to 0.376 fmol/mg; $P=0.014$). Similar changes were observed in the medulla, but the differ-
ences were statistically not significant (Figure 8B). The correlation between cortical in vivo ($f_{\text{int}}$) and in vitro binding ($B_{\text{max}}$) was significant (Spearman $r=0.609$; $P=0.012$).

**Discussion**

The presented data show increased radioligand binding to the AT$_1$R in hypoperfused pig kidneys resulting from hemodynamically significant renal artery stenosis. Although activation of the renin-angiotensin system was not measured, a rise in arterial blood pressure signified the presence of RVH. In the presented experiments, receptor binding was assessed both in vivo and in vitro in the pig renal cortex. The anatomy and physiology of the pig kidney are well characterized and show great similarities with humans. For example, in 1 rat model of RVH, glomerular angiotensin receptors were reduced in the clipped kidney 7 days postoperatively. In another rat model, AT$_1$R gene expression in both the clipped and contralateral kidney was upregulated in chronic but not in the acute phase of RVH. It is very difficult to study the angiotensin receptor in multiple species with comparative, quantitative methods. One problem is rapid activation or degradation of the system during the steps of tissue extraction and purification. There is simultaneous production and degradation of renin and Ang II by the kidney tissue. The above 2 rat studies and other published studies were all conducted at the tissue level using in vitro analysis technology. Our study is the first one that uses noninvasive imaging for studying the regulation of renal AT$_1$R in vivo, which may be the explanation for some of the differences from published data.

Factors that may be attributed to upregulation of the AT$_1$R in stenotic kidneys may include reduced renal perfusion pressure and altered local Ang II or other tissue hormones. Upregulation of AT$_1$R by itself creates a state of “hypersensitivity” to circulating and local Ang II. Nevertheless, regulation of the AT$_1$R in patients with chronic renal ischemic disease can be much more complex. This novel imaging method could help elucidate those changes and aid patient selection for the most appropriate method of revascularization or medical therapy.

In the presented simple but well-defined renal artery stenosis pig model, we have documented increased AT$_1$R binding in the hypoperfused kidney, an effect that was not abolished by the ACE inhibitor lisinopril, although lisinopril did attenuate hypertension. There is no simple explanation for this important observation. ACE inhibitors not only decrease the formation of Ang II but also reduce the degradation of bradykinin, which is a powerful vasodilator and a potential activator of AT$_1$R expression. Furthermore, intrarenal RAS is a more important receptor modulator than plasma Ang II. In the kidney, Ang II may be generated through ACE-independent pathways, such as chymase, leading to incomplete blockade of the formation of Ang II by AT$_1$R, the so-called “ACE-escape” phenomenon.

The significant correlation between the perfusion ratios determined with the 2 radiopharmaceuticals encourages one to use accumulation of $[^{11}C]KR31173$ during the first 1 to 2 minutes for assessment of organ perfusion. At this point, no attempt was made for development of a full-scale compartmental radioligand kinetic model. The impulse response function was calculated by deconvolution analysis, and $f_{\text{int}}$ derived from it was used to assess receptor-ligand binding. Deconvolution analysis eliminated the variance effects of injection technique, radioligand metabolism, and radioligand recirculation, whereas the retention ratio removed the effect of variable organ perfusion.

The regions of interest technique that was applied for derivation of TACs is applicable if one assumes homogeneous receptor distribution in the cortex. In fact, cortical radioligand binding in vivo is dominated by endothelial binding in the glomeruli and other blood vessels. The image resolution of PET, however, is not sufficient to analyze TACs of the renal medulla with predominantly tubular-epithelial binding. Application of advanced signal decomposition techniques that have been developed for analysis of brain receptor PET studies may help signal separation from these 2 renal structures. Autoradiography supports the hypothesis that AT$_1$R binding is upregulated in the renal cortex under conditions of reduced blood flow. The fact that, under in vitro conditions, medullary binding was higher than cortical binding, whereas under in vivo conditions cortical binding was higher, does not invalidate confirmation of this primary hypothesis.

In summary, the presented experiments found decreased accumulation but increased binding of $[^{11}C]KR31173$ consistent with reduced blood flow and increased AT$_1$R in the stenotic kidneys of pigs. Upregulation of receptor binding was confirmed by autoradiography and was not attenuated by lisinopril. Consequently, AT$_1$R PET may assist the molecular diagnosis of RVH and provide pivotal information pertinent to the initiation and progression of renal injury and repair under in vivo conditions.

**Perspectives**

In this study we applied an established porcine model of RVH to investigate the regulation of the renal AT$_1$R in response to reduced organ blood flow. PET documented increased AT$_1$R binding in renal hyperperfusion, an effect that was not abolished by lisinopril. Increased in vivo binding was confirmed by increased in vitro binding measured by quantitative autoradiography. These findings provide more insight in the biology of the kidney and support the concept of using AT$_1$R PET as a diagnostic biomarker of renovascular disease. Further studies should investigate the effect of reperfusion and confirm findings...
in humans. Prediction and follow-up of early tissue responses will be critical in the future to determine the best therapy option for individual patients with RVH and spare patients the cost and danger of ineffective therapies. We envision that noninvasive PET imaging of the renal AT$_1$R will ultimately lead to a rational approach to aid patient selection for revascularization or medical treatment, to monitor organ recovery, and to determine treatment success or failure at an early stage.

Acknowledgments
We are grateful to Dr Sung-eun Yoo, Center for Biological Modulators/Korean Research Institute of Chemical Technology Daejeon, Korea. We also are grateful to Dr Robert C. Speth, Department of Pharmacology, University of Mississippi, who provided [${}^{11}$C]KR31173. We are grateful to Dr Sung-eun Yoo, Center for Biological Modulators/Korean Research Institute of Chemical Technology Daejeon, Korea.

Sources of Funding
This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant R01 DK01813, Interdisciplinary Small Animal Imaging grant 5R24CA092871, and the Center for Biological Modulators/Korean Research Institute of Chemical Technology Daejeon, Korea.

Disclosures
None.

References


Positron-Emission Tomography Imaging of the Angiotensin II Subtype 1 Receptor in Swine Renal Artery Stenosis

Jinsong Xia, Esen Seckin, Yan Xiang, Melin Vranesic, William B. Mathews, Kelvin Hong, David A. Bluemke, Lilach O. Lerman and Zsolt Szabo

_Hypertension_. 2008;51:466-473; originally published online January 2, 2008; doi: 10.1161/HYPERTENSIONAHA.107.102715

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 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/51/2/466

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/