First D₁-like receptor PET imaging of the rat and primate kidney: implications for human disease monitoring

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Chronic kidney disease exists in 10% of the adult population in the United States and has become a major public health concern and financial burden on the healthcare system (25). The kidneys are largely responsible for controlling important measures of health such as blood pressure and plasma ion concentration, and dysfunction is associated with significant comorbidity including hypertension, diabetes, and cardiovascular disease (5a, 25). Hypertension is the most common chronic medical problem, with a total cost of more than $76 billion to the US economy. Most hypertension is essential (i.e., without a known cause) and a significant number of hypertensive individuals do not respond adequately to current antihypertensive therapies (19).

Several distinct dopamine receptor subtypes exist within two families: D₁-like include D₁ and D₅ receptors, and D₂-like contain the D₂, D₃, and D₄ subtypes. Dopamine signaling is important for the normal functioning of the kidney, and the dysfunction of dopamine receptors has been identified in hypertensive animal models (3, 12). It is estimated that 60% of sodium excretion during blood volume expansion is due to the effect of intrarenal dopamine (11). The action of intrarenal dopamine receptors has been leveraged in managing hypertensive crisis and acute kidney damage in humans with exogenous dopamine and the D₁-like agonist fenoldopam, and can actually improve kidney function in a variety of conditions (13, 15, 22, 24).

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Presently, few imaging tools are available to study metabolic and biochemical processes in the kidney of living animals. Imaging of the kidney is largely limited to anatomic visualization; some radiologic methods exist to estimate glomerular filtration rate, but are expensive and impractical. MRI has been the sole domain for functional renal imaging; however, many quantitative measurements require the use of contrast agents that are contraindicated in renally compromised individuals. Recently, a number of fMRI techniques have become viable options for measuring renal perfusion with no or little contrast. Blood-oxygen level-dependent fMRI measures the concentration of oxygen bound to hemoglobin, and this has been performed in kidneys using echo-planar imaging as well as the newer multiple gradient-recalled-echo sequences (18). Arterial-spin labeling requires no contrast, but produces an extremely noisy signal in the kidneys (23). Generally, fMRI methods are unable to provide real-world values but can be used to describe relative changes in renal perfusion (23).

Positron emission tomography (PET) facilitates in vivo imaging of biochemical processes noninvasively by measurement of a radiolabeled bioactive compound that is injected into the subject. PET is utilized clinically with 18-fluoro-deoxyglucose (FDG) to locate tumors and characterize neurologic and neuropsychiatric diseases. FDG has found some use in...
identification of renal masses, although it is often unnecessary with anatomic imaging such as CT and MRI (16). PET is now used routinely in translational research in humans with a variety of radiotracers and biological targets, such as diprenorphine for opioid receptors, DASB for serotonin transporters, and NNC 112 for dopamine (7, 9, 21). Given the high concentration of dopamine receptors in the kidney and the lack of quantitative methods to measure their activation in live animals, we sought to adapt $[^{11}C]\text{NNC 112}$ imaging, which has been used to study the human brain, into a tool that could be readily applied to kidney function and dysfunction as it relates to disease in humans.

To warrant investigation of D$_1$-like kidney imaging in humans, we evaluated $[^{11}C]\text{NNC 112}$ in rodents and in a nonhuman primate. NNC 112 exhibits highly specific and saturable binding within the renal cortex consistent with the high expression of D$_1$-like receptors. Using PET imaging we are able to show for the first time a significant effect of antihypertensive treatment on D$_1$-like binding potential in normotensive living rats. As alterations in the binding potential of intrarenal dopamine receptors have been associated with the development of kidney disease, PET imaging with $[^{11}C]\text{NNC 112}$ is particularly well suited to further explore the functional differences of dopamine metabolism in the healthy and pathologic kidney. $[^{11}C]\text{NNC 112}$ is already used in human brain studies; therefore, this method can be translated immediately to study renal dysfunction and hypertension in patients.

**MATERIALS AND METHODS**

**Animals.** All rodent work was conducted under the approved guidelines as defined by the Massachusetts General Hospital Institutional Animal Care and Use Committee (IACUC; protocol #0410–03-13). Rats were permitted food and water ad libitum and cared for by trained veterinary staff. Adequate anesthesia was maintained to alleviate stress induced by PET scanning, as monitored by toe pinch and tail flick response. Nonhuman primate work was conducted in accordance with the Massachusetts General Hospital IACUC. Measures taken to reduce stress in nonhuman primates include frequent feeding, social housing, enriching environments, and adequate use of anesthesia and analgesia during and after PET scanning procedures. Nonhuman primate imaging is conducted at intervals greater than 1 mo per animal to minimize suffering. Care was provided by a trained veterinarian, and no baboons were killed for the purposes of these experiments.

**Production of $[^{11}C]\text{NNC 112}$.** $[^{11}C]\text{CO}_2$ was produced through the $^{14}\text{N}(p,\alpha)^{11}\text{C}$ nuclear reaction in an Eclipse 11-MeV cyclotron (Siemens) and was reduced to $[^{11}C]\text{CH}_4$ using a TRACERlab FX-MeI unit (GE Healthcare). $[^{11}C]\text{CH}_4$ was trapped in a TRACERlab FX-M reactor (GE Healthcare) preloaded with a solution containing $1 \pm 0.2$ mg (+)-desmethyl-NNC 112 (Huayi Isotopes) and $<1$ mg K$_2$CO$_3$ (Sigma) in 0.3 ml anhydrous DMSO (Acros) at 20°C. The solution was heated to 120°C for 3 min and then quenched with 1.2 ml HPLC mobile phase (66% 0.1 M ammonium formate in H$_2$O, 34% MeCN). The reaction mixture was purified by reversed-phase semi-preparative HPLC with an elution time of 9–10 min. The desired fraction was collected, diluted with 20 ml H$_2$O, and loaded onto a C18 Sep Pak Plus (Waters). After the Sep Pak was washed with water, the product was eluted with 1 ml EtOH and 9 ml injectable saline ( Hospira) and filtered through a 0.2-μm sterile filter (Millex).

**Rat PET measurement with unlabeled NNC 112 and SCH-23390 pretreatment.** All rat measurements were conducted in a Concorde P4 primate scanner (Concorde Microsystems, Knoxville, TN). Rats were arranged on a cardboard tray containing nosecones connected to medical grade oxygen (2 l/min) and containing vaporized isoflurane (2–3%, Forane, Baxter Pharmaceuticals, Deerfield, IL). The large bore of the P4 scanner permitted us to scan up to four rats simultaneously.

Intravenous access and drug delivery were achieved by tail vein catheterization. Scanning was initiated 5 min following drug or vehicle delivery, and just before injection of the radiotracer. Raw PET data were recorded in list-mode for 80 min and reconstructed using a filtered back-projection algorithm to produce DICOM images suitable for analysis. Attenuation correction was possible after a 15- to 30-min transmission scan using a $^{60}$Ge point source.

In the first set of experiments, self-saturation was assessed using four rats paired into baseline or pretreatment. In the first pair, one rat received intravenous delivery of a vehicle and the other rat received intravenous unlabeled NNC 112 (2 mg/kg). The second pair was separated into intravenous vehicle and intravenous unlabeled NNC 112 (2.5 mg/kg) in the same vehicle. Finally, a single rat was administered SCH-23390 (30 μg/kg), a selective D$_1$-like receptor antagonist ($K_I = 0.2$ nM) to assess specific binding (5).

**Rat PET measurements with losartan pretreatment.** Scanning procedure and reconstruction were identical to that of self-blocking experiments in rats, except that four rats were arranged inside the bore of the scanner and data were collected on all of them simultaneously. One group ($n = 4$) received 15 mg/kg losartan ip 30 min before the scan, and the second group ($n = 4$) received the same dose of losartan 60 min before the start of scan. Rats were administered 9.74 ± 0.2 MBq of $[^{11}C]\text{NNC 112}$ intravenously as a slow bolus after scanner acquisition began.

**Papio anubis MR-PET measurement with SCH-23390.** A 15.1-kg male Papio anubis baboon, deprived of food 12 h before the study, was induced into anesthesia with intramuscular ketamine (10 mg/kg). Anesthesia was maintained with isoflurane (1.5–2%, Forane, Baxter Pharmaceuticals) delivered via endotracheal intubation. An anestetial vein catheter and radial artery catheter were placed by a trained veterinarian for drug delivery and blood collection for metabolite analysis. The baboon was positioned inside of a Biograph mMR scanner (Siemens, Munich, Germany) with a PET resolution of 5 mm and field of view of 59.4 cm and 25.8 cm (transaxial and axial, respectively) capable of collecting MRI data simultaneously with a 3T magnet. Eighteen blood samples were collected within the first 3 min after pretreatment with losartan. Arterial input function, followed by blood collection for metabolite analysis at 5-, 10-, 20-, 30-, 45-, 60-, and 80-min time points. Dynamic data were reconstructed using a 3D-OSEM method resulting in a full width at half-maximum (FWHM) value of 4 mm. Attenuation correction was MRI-based and used a proprietary Siemens algorithm.

Two scans were completed in the same baboon. Before the first scan, a vehicle consisting of normal saline was injected and scanning was initiated 5 min later. An intravenous preparation containing 146.9 MBq of $[^{11}C]\text{NNC 112}$ was injected and acquisition proceeded for 80 min. A second scan was performed in the same baboon immediately following the baseline scan. Five minutes before the scanning sequence was initiated, 30 μg/kg of SCH-23390, a potent and selective D$_1$ antagonist ($K_I = 0.2$ nM), were delivered to verify target engagement and characterize specific binding in the kidney (5). Again, scanning was initiated before injection of 22.09 MBq of $[^{11}C]\text{NNC 112}$ followed by blood sampling.

**Plasma and metabolite analysis.** Plasma radioactivity and metabolite analysis were performed as outlined in previous work (8). Arterial samples collected during imaging from the baboon were centrifuged to obtain plasma, which was then removed and placed in an automated gamma counter that was calibrated to the PET scanner. Metabolite analysis was conducted on a custom-automated robot fitted with Phenomenex SPE Strata-X 500-μg solid phase extraction cartridges that were primed with ethanol (2 ml) and deionized water (20 ml). Protein precipitation was achieved by addition of plasma (300 μl) to acetonitrile (300 μl), which was centrifuged for 1 min to obtain protein-free plasma (PFP). Three hundred microliters of PFP/aceto
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nitrile solution were diluted into deionized water (3 ml), loaded onto the C18 cartridge, and removed of polar metabolites with 100% water. Next, a series of extractions were performed using water and acetonitrile in quantities: 2 × 95:5, 90:10, 85:15, 80:20, and 100% acetonitrile at a volume of 4 ml. Each sample was counted in a WIZARD2 Automatic Gamma Counter to determine the presence of radiolabeled metabolites.

PET image analysis. All data were imported to PMOD (PMOD Technologies) as DICOM files. These images were coregistered and saved in NIFTI format. Organs were selected as spherical or ellipsoid volumes-of-interest on anatomic T2 weighted MRI scans for baboon data. Rat ROIs were drawn on an averaged PET image containing the first 30 min of scanning. Cortex was isolated from the renal pelvis by proceeding dorsally through coronal slices until renal pelvis was no longer visible and manually drawing an ROI. Care was taken to ensure that each ROI had a radius equal or greater to the FWHM resolution of the respective scanner to minimize partial volume effects. Activity data were then expressed as %ID/cc (%injected dose per ml of tissue) for export from PMOD. To create a standardized uptake value (SUV) in rat, time frames from 24–60 min were averaged to produce a mean radioactivity that was normalized to injected dose and body weight. Baboon standardized uptake values were calculated identically using mean radioactivity from 20–80 min after [11C]NNC 112 injection. Volumes of distribution were calculated using the PMOD kinetic analysis module. Plasma activity and radioactivity were fitted to a 3-exponential model to derive an input function for the injection of the radio-tracer. The resulting metabolite-corrected plasma radioactivity was utilized to calculate a VT by graphical analysis (20).

RESULTS

Rat: [11C]NNC 112 PET saturation experiments with unlabeled NNC 112. For baseline scans, rats were administered a vehicle composed of normal saline to control for potential formulation effects of the preadministered blocking dose on radioactivity pharmacokinetics and accumulation. In the baseline studies, radioactivity in the left renal cortex reached an average of 0.36% of the injected dose per ml of tissue at 35 min. In rats pretreated with 2 or 2.5 mg/kg unlabeled NNC 112, radioactivity in the left renal cortex reached an average of only 0.083% injected dose per ml of tissue at 35 min corresponding to a blocking percentage of ~77% (Fig. 1). Early time points were dominated by radioactivity in the lungs most likely reflecting their large volume of blood. Radioactivity in the renal cortex peaked at 2 min, and it appears uniform and consistent with anatomic separation between the renal cortex and pelvis. While radioactivity in the renal cortex did not change appreciably after reaching equilibrium at 30 min, in the renal pelvis it increased throughout the duration of the scan likely reflecting the metabolism and partial urinary excretion mechanism of NNC 112 (6). Later time points show radioactivity accumulating in the gut lumen that was not altered by pretreatment.

Papio anubis: [11C]NNC 112 PET renal biodistribution and blocking study. In [11C]NNC 112 imaging experiments in Papio Anubis (Fig. 2), left renal cortex radioactivity reached an approximate steady state by 50 min in both baseline preadministration (30 µg/kg SCH-23390) scans. At 60 min post [11C]NNC 112 administration to baboon, left renal cortex radioactivity reached 0.126 and 0.051% ID/ml in baseline and pretreatment with SCH-23390, respectively, for a blocking percentage of ~60%. It should be noted that this dose was selected from the literature, as it is not expected to fully saturate D1-like receptors in baboon. Left renal cortex volumes of distribution for [11C]NNC 112 in baseline and pretreatment were 121.8 and 8.4 ml·ccm⁻¹ (93% reduction). Renal cortex radioactivity reached equilibrium at 50 min, with renal pelvis radioactivity increasing throughout the duration of the scan reflecting urinary excretion of [11C]NNC 112. Pretreatment with SCH-23390 did not alter renal pelvis radioactivity curves, although the gallbladder exhibited much greater radioactivity with pretreatment. The intestinal lumen, as in rat, accumulated radioactivity that was not affected by pretreatment.

[11C]NNC 112 binding in losartan-pretreated rats. Untreated Sprague-Dawley rat controls were utilized from the previous saturation studies. Rats pretreated with 15 mg/kg losartan intraperitoneally showed no obvious physiological response to the drug or altered response to isoflurane anesthesia, and they had no change in morbidity following PET acquisition. Results showed (Fig. 3) that in general, right renal cortex displayed greater uptake at later time points that is better correlated with liver uptake of [11C]NNC 112 metabolites and therefore all values were calculated using only the left renal cortex. Rats that received only saline pretreatment had an SUV of 1.051 at 29.5 min. Following an acute single dose of losartan 30 min before injection of [11C]NNC 112 and start of acquisition, renal D1-like increased by 31% to an average SUV of 1.376 (SD = 0.091, P = 0.025). Renal D1-like binding collectively returned to that of drug naive animals at 1 h (SUV = 1.076, SD = 0.171, P = 0.878), although one rat in this group continued to exhibit elevated binding potential (SUV = 1.266).

DISCUSSION

Rat saturation experiments. Baseline PET imaging of the rat demonstrated binding of [11C]NNC 112 in the renal cortex, a region of the kidney known to express D1-like receptors. Very little radioactivity was observed in the renal pelvis at early time points in accordance with its deficit of dopamine receptors, although it did accumulate radioactivity throughout the duration of PET imaging reflecting the urinary excretion of [11C]NNC 112. Right kidney radioactivity was uniformly greater than left kidney, although this was more associated with the physical proximity and accumulated radioactivity in the liver. To minimize this potential confound, we utilized only the left kidney for quantification of renal uptake. A blocking percentage of 77% in rats pretreated with unlabeled NNC 112 suggests that the image is dominated by specific binding of
[11C]NNC 112. Consistent with the urinary excretion of [11C]NNC 112 metabolites, it may not be possible to entirely block uptake within the cortex as some of the reported radioactivity will be from filtration and excretion into the nephron rather than specific binding.

**Papio anubis saturation experiments.** As in rat, intravenous administration of [11C]NNC 112 demonstrated uptake in the renal cortex in accordance with its expression of D1-like receptors. There was no initial uptake in the renal pelvis, although it began to accumulate radioactivity as [11C]NNC 112 and radiolabeled metabolites were excreted. Radioactivity was significantly decreased in the renal cortex by pretreatment with SCH-23390. Initially high radioactivity in the lungs reflects the large volume of blood they contain, and no other organ showed significant uptake (specific binding) reflecting the sparse expression of D1-like receptors. Gallbladder and liver maintained the highest radioactivity second to the kidneys, consistent with the known NNC 112 primary excretion route into the bile. No significant changes were observed in regions other than kidney, suggesting the radioactivity in these regions was reflective of nonspecific binding. The gall bladder, however, accumulated greater radioactivity with pretreatment. This is most likely a result of increased metabolism and excretion of blood-based [11C]NNC 112 as the previously available D1-like receptors were occupied by SCH-23390. With the large reduction in renal cortex radioactivity from pretreatment with a D1/D5 antagonist, we showed that the image is not altered by competitive inhibition of metabolism or urinary excretion as may have been the case in self-blocking experiments.

**Altered dopamine binding in losartan-treated rats.** Acute pretreatment at 30 min with losartan elicited a uniform increase in D1-like binding potential in the rat left renal cortex consistent with previously reported autoradiography data (17). The effect had nearly disappeared after an hour, with only one animal retaining slightly elevated [11C]NNC 112 uptake. As each animal was losartan naive, the disappearance of altered dopamine binding may be consistent with the 3- to 6-wk latency period before an antihypertensive effect is observed with losartan; the role of this effect in the eventual antihypertensive effect of angiotensin receptor blockers is as of yet unknown (1). Additionally, the heterogeneous effect on dopamine binding at 1 h may be representative of the high rate of therapeutic failure with antihypertensive drugs. However, due to the nature and small size of the pilot study, this remains unclear.

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![Fig. 2](image-url)

Conclusion. Baseline scans in both rat and Papio anubis demonstrate binding of [11C]NNC 112 in regions of the kidney known to express D1-like receptors. Additionally, a lack of specific binding in other organs is consistent with the known distribution of these receptors in rat and baboon. The high blocking percentage in rats pretreated with unlabeled NNC 112 suggests that the image was dominated by specific binding of [11C]NNC 112. Consistent with the urinary excretion of [11C]NNC 112 metabolites, it may not be possible to entirely block uptake as some of the reported radioactivity will be from excretion rather than specific binding. Blocking experiments with SCH-23390 in the baboon showed target engagement at D1-like receptors. Additionally, since there is a 60% reduction in radioactivity in the renal cortex from a dosage of SCH-23390 that is not expected to fully saturate D1-like receptors, we showed that the image is not altered by competitive inhibition of metabolic or excretory physiology as may have been the case in self-blocking experiments with unlabeled NNC 112. Therefore, we conclude that the images produced by [11C]NNC 112 in the renal cortex in both rat and baboon are predominantly a reflection of specific binding to D1-like receptors and not excretion or nonspecific binding. The pilot study with losartan pretreatment supports this paradigm of renal PET imaging with [11C]NNC 112 in healthy and disease-model animals. With a small cohort of study animals, we were able to identify a meaningful increase in D1-like binding potential resulting from modulation of the angiotensin system. The ability of renal PET to replicate data from autoradiographic studies lends support for external validity while also highlighting the potential benefits of noninvasive imaging over existing methods: ability to perform longitudinal studies, nondestructive study of physiology, and the possibility to safely utilize human subjects.

Future prospects. The noninvasive nature of PET and efficacy of NNC 112 at measuring the binding potential of D1-like receptors will permit the first human studies targeted at elucidating the contribution of renal dopamine dysfunction to kidney disease using imaging. Central to these studies would be measurement and comparison of D1-like binding potential in healthy normotensive individuals and patients with kidney dysfunction. Divergence from a standardized binding potential among healthy individuals may then be correlated with the presence or severity of kidney dysfunction in sick individuals. With the ability of renal PET to be readily applied to living human subjects, it will be possible to further study the correlation between hypertension and altered renal dopamine. Additionally, the role of current antihypertensives on renal dopamine can be measured with [11C]NNC 112. While it has been established that therapies targeting angiotensin can alter the function of renal dopamine receptors in ex vivo models, in vivo measurement is now possible for angiotensin as well as other antihypertensive targets (e.g., beta blockers, calcium channel blockers, loop diuretics). With the high prevalence of treatment-resistant hypertension, renal PET may be used to directly probe dopamine receptors for a greater understanding of their role in therapeutic failure. Similarly, measuring the engagement of kidney receptors may have some implications for drug design and predicting efficacy in specific patients. With the increasing availability of multifunctional MRI/PET scanners, it will be possible for researchers to simultaneously measure dopamine receptors and kidney perfusion or function. This will permit the derivation of a relationship between receptor occupancy and physiologic effect, which would be well-suited for determining drug dosages that have optimal effect for a range of therapeutic goals.

We showed that in vivo measurement of renal D1-like receptors is currently possible using PET imaging with [11C]NNC 112 in rat and nonhuman primate. The large healthcare burden associated with hypertension and kidney disease represents the necessity for research tools to better understand the underlying biochemistry that leads to physiologic and pharmacologic failure. With the well-established safety profile of NNC 112 for use in humans and evidence for utility in measurement of renal dopamine in healthy and pathologic states, we believe that PET imaging using [11C]NNC 112 can be readily applied to the study of kidney disease in human subjects.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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