Positron Emission Tomography Studies on angiotensin II subtype 1 receptor (AT_1R)

Doctoral thesis

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INTRODUCTION

Positron Emission Tomography (PET) has enjoyed tremendous growth in recent decades in research and clinical practice internationally, as well as in Hungary. Unlike Computer Tomography (CT) and Magnetic Resonance Imaging (MRI), which can visualize the anatomical structures of the body, PET reveals the metabolic changes occurring in the living body on a cellular level, giving more information on physiological and pathological changes. Blood perfusion, glucose metabolism, oxygen uptake and receptor binding can be measured by PET. The spread of the cyclotrons in the last one and a half decades made short-lived radionuclides widely available. With the modern isotope techniques almost every organic molecule can be labeled with positron emitters, helping to study various receptor-ligand interactions.

The renin-angiotensin-aldosterone system (RAAS) and angiotensin II are important parts in the regulation of extracellular fluid volume and the cardiovascular system. PET helps to study this system in a very new dimension.

Between April 2002 and October 2004 I was a postdoctoral fellow at the Johns Hopkins University (JHU) in Baltimore, Maryland. I had the privilege to learn the basics of PET technique and to join the research group of Professor Zsolt Szabo to study angiotensin II subtype 1 receptor (AT_1R) at the PET Center of JH Medical Institutions.

In the first part of my thesis I will introduce the [^{11}C]KR31173, a new AT_1R specific radiotracer developed by our group. I will show its AT_1R specific binding by *ex vivo* studies (biodistribution and pharmacology) and its applicability for *in vivo* small-animal and clinical PET studies using mice, dogs and baboon.

In the second part I will demonstrate in animal models the advantages of PET in the study of RAAS. For this we applied [^{11}C]L-159,884, another AT_1R specific radioligand developed earlier and used in many animal studies. ACE inhibitors are important drugs in the treatment of cardiovascular diseases. I will demonstrate *in vivo* with PET that acute or chronic ACEI administration in dogs will change AT_1R radioligand binding. Finally I will show the results of our PET studies in two-kidney-one-clip experimental (Goldblatt) hypertension of dogs.

AIMS

1. AT_1R binding characteristics and PET imaging of [^{11}C]KR31173

1.1 *Ex vivo* biodistribution and pharmacological studies for AT_1R specific binding of the [^{11}C]KR31173 radioligand with the original radiosynthesis in mice

Our hypothesis:

- [^{11}C]KR31173 with the original radiosynthesis shows high accumulation and specific binding in the organs of mice rich in AT_1R

1.2 *Ex vivo* biodistribution and pharmacological studies for AT_1R specific binding and selectivity of the [^{11}C]KR31173 radioligand with the improved radiosynthesis in mice

Our hypothesis:

- [^{11}C]KR31173 with the improved radiosynthesis shows high accumulation, highly specific and selective binding in the organs of mice rich in AT_1R

1.3 Small-animal PET imaging of [^{11}C]KR31173 in mice

Our hypotheses

- [^{11}C]KR31173 is suitable for small-animal PET imaging in mice
- The PET imaging studies show high AT_1R specific binding of [^{11}C]KR31173 in mice.

1.4 Clinical PET imaging of [^{11}C]KR31173 in dogs

Our hypotheses:

- [^{11}C]KR31173 is suitable for clinical PET imaging of dogs.

- The PET imaging studies show high AT_1R specific binding of [^{11}C]KR31173 in dogs.

1.5 Clinical PET imaging of [^{11}C]KR31173 and [^{11}C]L-159-884 in baboon

Our hypothesis:

- [^{11}C]KR31173 is suitable for clinical PET imaging of baboon.
- The PET imaging studies show high AT_1R specific binding of [^{11}C]KR31173 in baboon.
- [^{11}C]KR31173 shows higher activity and specific binding in the baboon renal cortex than [^{11}C]L-159-884.

1.6 Metabolism, plasma protein binding and urinary excretion of [^{11}C]KR31173 and [^{11}C]L-159-884

Our hypotheses:

- The metabolism of [^{11}C]KR31173 is significantly decreased with the new radiosynthesis method.
- The plasma protein binding of [^{11}C]KR31173 is lower in baboon and human plasma than of [^{11}C]L-159-884.

2. Investigation of the effects of acute and chronic angiotensin converting enzyme inhibitor (ACEI) treatment on the AT_1R density in the dog kidney

The present study was designed to elucidate the effect of acute and chronic angiotensin converting enzyme inhibitor (ACEI) treatment on the *in vivo* binding of radioligand [^{11}C]L-159,884 to the AT_1R in the dog kidney. Since ACEI therapy is broadly used for treating patients with cardiovascular diseases, the results of the study are important for the possible human AT_1R PET imaging in the future.

Our hypotheses:

- Chronic ACEI treatment upregulates the AT_1R, which is reflected in increased binding of the AT_1R specific radioligand [^{11}C]L-159,884.
- Acute ACEI treatments will result in changes of receptor binding that will be less marked than the changes observed with chronic treatment.

3. Investigation of the effects of experimental renovascular hypertension on the AT_1R density in the kidney of dogs

The present goal of our study was to investigate the experimental Goldblatt-type 2K, 1 C hypertension, and to study *in vivo* the changes of AT_1 receptor density in the dog kidney with PET. Our long-term goal is the application of human AT_1R PET imaging for the diagnosis of RVH.

Our hypotheses:

- In experimental 2K, 1 C hypertension the *in vivo* PET AT_1R binding parameters are increased in the hypoperfused dog kidney.
- The increased *in vivo* PET AT_1R binding parameters correlate positively with arterial blood pressure and correlate negatively with kidney perfusion.

MATERIALS AND METHODS

Ex vivo biodistribution and pharmacological studies in mice

In the biodistribution studies healthy male CD-1 mice (n=3/group; JHU Animal services) were used and were injected via tail vein with equivalent amount of radiotracer (10-20 MBq) in equivalent volume (0.2 ml). The animals were sacrificed by cervical dislocation at 5, 15, 30, 60, and 90 minutes after injection. The organs (brain,

heart, lungs, liver, kidneys, and adrenal gland) were weighed and the tissue radioactivity measured with an automated gamma counter (LKB Wallac 1282 Compugamma CS Universal Gamma Counter). The percent- injected dose per gram of tissue (%ID/g) was calculated.

In the pharmacological studies the animals were pretreated by cold antagonists (10 min prior to radioligand administration by iv injection, 1 mg/kg; 30 min prior to radioligand administration by intraperitoneal injection, 2 mg/kg) or saline. 60 min after radioligand injection the animals were sacrificed and the organ activity were measured as in the biodistribution studies.

In the pharmacological studies AT_1 antagonist MK-996, unlabeled KR31173 SK-1080 and AT_2 antagonist PD123,319 were used. SK-1080, unlabeled KR31173 and its protected precursor were provided by Korean Research Institute of Chemical Technology (Taejon, South Korea). MK-996, unlabeled L-159,884 and its precursor were the gifts of Merck Research Laboratories (West Point, PA), PD-123,3l9 was provided by Parke-Davis Pharmaceutical Research Division (Ann Arbor, MI).

Small animal PET studies in mice

Healthy male CD-1 mice (JHU Animal services) were imaged with an „ATLAS" (Advanced Technology Laboratory Animal Scanner, National Institutes of Health, Bethesda, MD) small animal PET scanner. The animals were premedicated with a mixture of ketamine–acepromazine–saline (Sigma-Aldrich, St. Louis, MO; 1:1:2; 50 μl/22 g, subcutaneous), and anesthesia was maintained with isoflurane 0.5–1.0% at 0.4–1.0 L/min (Halocarbon Products Corp., River Edge, NJ). After the injection of the [^{11}C]-labeled radioligand via the tail vein, a dynamic PET study was performed for 60 min with the following image sequence: four 15s images, three 1-min images, three 2-min images, six 5-min images and two 10-min images.

PET imaging of dogs and baboon

PET imaging and animal preparation

PET studies were performed with a GE Advance PET scanner (GE Medical Systems, Milwaukee, WI), which has an average inplane-crossplane spatial resolution of 5,4 mm and 5,8 mm. A transmission scan with a 370 MBq Germanium-68 pin source was obtained first and was used subsequently for attenuation correction of the PET scans.

The beagle dogs (JHU Animal services) were premedicated with acepromazine (0.2 mg/kg im; Sigma-Aldrich, St. Louis, MO) and were anesthetized intravenously with sodium pentobarbital (25 mg/kg; Sigma-Aldrich, St. Louis, MO). Additional pentobarbital was administered during the imaging study at a rate of 3 mg/kg per hour. The animals were intubated during the PET imaging study. Heart rate, blood pressure and oxygen saturation were monitored continuously. One femoral artery line was placed to obtain the arterial input function. Two peripheral venous lines were placed for fluid replacement, anesthesia administration and tracer injection. PET imaging was performed for 95 min with the following protocol: four 15s images, three 1-min images, three 2-min images, three 5-min images, three 10-min images and two 20-min images.

Anesthesia was induced in one male *Papio anubis* baboon (24 kg; JHU Animal services) by intramuscular injection of 9 mg/kg Saffan (Pitman-Moore, Middlesex, UK) and was maintained with intravenous (iv) infusion of Saffan at a rate of 7 mg/kg per hour. The animal was monitored as described at the dogs. PET imaging was performed for 75 min with the following protocol: four 15s images, three 1-min images, three 2-min images, three 5-min images, three 10-min images and one 20-min image.

PET image processing and quantification of radiotracer binding

In dogs and baboons, the PET images were reconstructed by filtered back projection using a ramp filter. The „Imager 3D" software (Johns Hopkins University, Baltimore, MD) was used for image reconstruction and to obtain time–activity curves

(tissue concentration curves), regions of interest were marked out around the organ. The following radioligand binding parameters were calculated:

- **Tissue activity** (Bq/ml/MBq ID)
- **Radioligand retention**: 75-95 min tissue activity divided by the maximal tissue activity.
- **Gjedde-Patlak's K_i**
- **Logan's total distribution volume (*DV*)**

For the purpose of compartmental modeling, 35 samples of 0.5 ml blood were obtained via the femoral artery line from the time of injection until the end of PET acquisition to create **the input function**. The input function was cross-calibrated with the PET scanner. Additional arterial blood samples (1 ml) were collected at six time points (5, 15, 30, 45, 60 and 90 min pi) to measure the unmetabolized tracer fraction using high-performance liquid chromatography (HPLC) of the separated plasma samples.

Hormone levels and in vitro receptor determination

- **Plasma renin activity (PRA)** (ng/ml/h) radioimmunoassay studies (GammaCoat Plasma Renin Activity ^{125}I RIA Kit; Diasorin, Stillwater, MN), PRA control reagent (Diasorin)).
- **Plasma Angiotensin II (Ang II)** (pg/ml) radioimmunoassay studies ([^{125}I]-Angiotenzin II RIA Kit; Phoenix Pharmaceutical, Belmont, CA) Ang II control reagent (Phoenix Pharmaceutical).
- **Plasma aldosterone** (pg/ml) radioimmuno-assay studies (Coat-A-Count Aldosterone; Diagnostic Product Corp., Los Angeles, CA), aldosterone control reagent (Lyphochek Hypertension Markers Control; Bio-Rad, Irvine, CA).
- ***In vitro* determination of AT_1R** Samples were incubated with increasing concentrations of ^{125}I-[Sar^1,Ile^8]Ang II (0.05-4 nM). The maximum number

of specific binding sites (***Bmax***) and ligand affinity (***Kd***) were calculated by Scatchard analysis of the radioligand saturation curves using the program Prism (Graph-Pad Software, San Diego, CA, USA).

Statistical calculations

Statistical tests were performed by the Statistical Package for the Social Sciences (SPSS)/Windows version 12 (SPSS. Chicago, IL) and in Microsoft Excel 2002 (Microsoft Corporation Seattle, WA).

RESULTS

1. AT_1R binding characteristics and PET imaging of [^{11}C]KR31173

1.1 *Ex vivo* biodistribution and pharmacological studies for AT_1R specific binding of the [^{11}C]KR31173 radioligand with the original radiosynthesis in mice

Normal, nonfasted, male CD-1 mice (26.0-30.8 g, n=15+9) were injected via the tail vein with 19.1 MBq (517 μCi; 1.45 μg/kg) of [^{11}C]KR31173. The radioligand showed high initial uptake in the liver, which rapidly washed out over 30 minutes. After 30 minutes, the highest uptake was in the adrenal glands and kidneys (9.39 ± 3.71 %ID/g and 7.12 ± 0.93 %ID/g at 60 min). The MK-996 (n=3) and KR31173 (n=3) AT_1 antagonists significantly inhibited the uptake of [^{11}C] KR31173 in the adrenal glands (91%), kidneys (90%), lungs (88%), and heart (92%).

1.2 *Ex vivo* biodistribution and pharmacological studies for AT_1R specific binding and selectivity of the [^{11}C]KR31173 radioligand with the improved radiosynthesis in mice

Healthy male CD-1 mice (weight range 27–31 g) were injected via the tail vein with 21 MBq (253 GBq/μmol) of [^{11}C]KR31173 produced by the improved radiosynthesis method. The animals were pretreated 30 min prior to radioligand administration by intraperitoneal injection of either saline (n=4), the AT_1R antagonist SK-1080 (n =4), or the AT_2R antagonist PD123,319 (n=4).

The tissue concentration was the highest sixty minutes after the injection of [^{11}C]KR31173 , in the known AT_1R rich organs, in the adrenals (27.3 ± 6.4 %ID/g) and the kidneys (11.3±1.0 %ID/g). Pretreatment with AT_1 antagonist SK-1080 significantly reduced (paired t-test; P=0.0001–0.01) accumulation of the radioligand in the adrenal

glands (98% inhibition), lungs (96% inhibition), heart (96% inhibition) and kidneys (82% inhibition). Pretreatment with AT_2 antagonist PD123,319 resulted in no statistically significant change of binding in the heart, lungs, kidneys, adrenals or liver.

1.3 Small-animal PET imaging of [^{11}C]KR31173 in mice

Healthy male CD-1 mice (n =13, 30–44 g) were used. Thirty minutes prior to radioligand administration, the animals were pretreated intraperitoneally with either saline (n =7) or 2 mg/kg of the AT_1R antagonist SK-1080 (n=6). An average dose of 13 MBq (345 GBq/μmol) of [^{11}C]KR31173 was injected.

PET scans obtained in mice demonstrated distinct radioligand accumulation in the kidneys: tissue activity was high in the control animal and was reduced in the animal pretreated with SK-1080. Liver activity was seen in the control animal, and it appeared higher than renal activity. After pretreatment with SK-1080, renal uptake of the radioligand was inhibited by 49±6 % at 10–60 min.

1.4 Clinical PET imaging of [^{11}C]KR31173 in dogs

PET imaging was performed in male beagle dogs (n=3, 15.5±0.5 kg). Subsequently, two dynamic PET studies were performed with [^{11}C]KR31173 set 135 min apart. The injected dose was 275 ± 58 MBq (113 GBq/μmol). During the second PET study the animal received 1 mg/kg of AT_1 antagonist SK-1080 injected intravenously 30 min prior to the radiotracer.

PET scans obtained 75–95 min pi showed distinct accumulation of [^{11}C]KR31173 in the canine adrenal and renal cortex. Neither the adrenals nor the renal cortex could be distinguished by PET imaging within this same time frame if the animal was pretreated with SK-1080. In the control animal, activity was also seen in the liver and was higher than the activity in the kidneys.

At 75–95 min pi, tissue activity of [^{11}C]KR31173 with the original radiosynthesis was 63±32 Bq/ml/MBq ID and was reduced by 48% after SK-1080 pretreatment. With the improved radiosynthesis of [^{11}C]KR31173, tissue concentration

was very similar at 75–95 min pi (63±14 Bq/ml/MBq ID), but specific binding was much higher, 92% as determined after AT_1R antagonist pretreatment. With both radiosynthesis techniques, the tissue concentration of [^{11}C]KR31173 in the canine renal cortex was lower than the tissue concentration published for [^{11}C]L-159,884

1.5 Clinical PET imaging of [^{11}C]KR31173 and [^{11}C]L-159-884 in baboon

In one male *Papio anubis* baboon (24 kg) an average of 345 MBq (291 GBq/μmol) [^{11}C]KR31173 and 530 MBq (243 GBq/μmol) [^{11}C]L-159-884 radioligand was injected.

[^{11}C]KR31173 resulted in considerably higher activity in the baboon renal cortex than [^{11}C]L-159,884 at 55–75 min pi (345 Bq/ml/MBq ID and 96 Bq/ml/MBq ID) Pretreatment with SK-1080 reduced the renal cortical concentration of [^{11}C]KR31173 by 81% and of [11C]L-159,884 by 34%.

1.6 Metabolism, plasma protein binding and urinary excretion of [^{11}C]KR31173 and [^{11}C]L-159-884

In dogs, the observed metabolism of [^{11}C]KR31173 significantly decreased when applying the new radiosynthesis method (from ~61% to 15% at 90 min, paired t-test; P=.0001). In the baboon, only the new method of synthesis was used, and resulted in 21% plasma metabolites 90 min after injection. In the plasma samples of dog, baboon and human, protein binding of [^{11}C]KR31173 was lower than [^{11}C]L-159,884. Urinary excretion of [^{11}C]KR31173 during 90 min pi was mostly unmetabolized and 9–12% in dogs and 12–18% in baboons.

2. Investigation of the effects of acute and chronic angiotensin converting enzyme inhibitor (ACEI) treatment on the AT_1R density in the dog kidney

Ten adult male beagles (average weight 13.8 kg) were studied. The animals were divided into a control group (n=5) and an ACEI treatment group (n=5), and were imaged on three different days with an average injected dose of 370 MBq [^{11}C]L-

159,884. The following schedule was applied in the animals of the ACEI group: On day 0, at the end of the 1- week acclimation period, a baseline PET study was performed in each dog. A second PET scan was performed on day 14 after acute administration of *enalaprilat* (*Vasotec IV*; Merck, North Wales, PA); the animals received 4 mg enalaprilat by slow intravenous infusion 30 min before [11C]L-159,884 administration and PET imaging. A third PET study was performed on day 28 after the animals had been treated chronically with *lisinopril* (*Prinivil;* Merck, North Wales, PA) for 2 weeks; lisinopril was administered orally by mixing 20 mg/day into their food. The same schedule (days 0, 14, and 28) was followed in control animals that were also fed with food but did not receive either acute or chronic ACEI therapies. On day 28, after the third PET scan, the animals were sacrificed by euthanasia for tissue harvesting.

Although group differences of PRA were significant with higher values measured in dogs on chronic ACEI (p=0.029; Mann-Whitney), there was no significant difference between the plasma Ang II and aldostreone levels of the treatment groups. In the ACEI-treated group tissue activity, retention, and Ki increased from day 0 to 28:

K_i changed from 0.021 to 0.027 (medians, p = 0.020; Wilcoxon); radioligand retention from 0.294 to 0.331 (medians, p = 0.030; Wilcoxon), and tissue activity from 77 to 117 Bq/ml/MBq ID-re (medians, p = 0.011; Wilcoxon). These radioligand binding parameters were increased after acute ACEI treatment, but only the increase of radioligand retention was significant (p=0.014; Wilcoxon). Increased ligand binding was confirmed by the higher Bmax values observed in glomeruli of treated animals (1,094 pmol/mg protein, median) compared to controls (919 pmol/mg protein, median, p=0.0458 Mann-Whitney). Scatchard analysis revealed that the differences in receptor binding were not affected by the binding affinity expressed as Kd.

3. Investigation of the effects of experimental renovascular hypertension on the AT_1R density in the dog kidney

Nine adult beagle dogs (12.3 ± 1.1 kg) were included in the study. All animals underwent digital subtraction angiography (DSA) for imaging the renal arteries. After laparotomy was performed by small flank incision, a 2.5 or 2.75 mm ameroid

constrictor (Research Instruments SW, OR) was placed around the left renal artery in 6 animals. In 3 sham-operated animals, the constrictor was placed around the left renal artery but was immediately removed.

Gadolinium (0.2 mmol/kg iv.) enhanced MRA was performed 1-4 weeks after placement of the ameroid constrictor with a General Electric CV/i magnetic resonance scanner. Renovascular hypertension developed in four dogs 1-4 weeks after placement of the ameroid constrictor. Two animals with ameroid constrictor did not develop RVH and were assigned to the control group, which already included 3 sham-operated animals. In the two animals of unsuccessful renal artery stenosis, MRA showed significant collateral flow while both MRA and [^{15}O]water PET showed symmetrical cortical blood flow.

PET studies were performed with a GE Advance scanner (GE Medical Systems, Milwaukee, WI) within 24 hours of the MRA. For comparison of the kidneys, renal perfusion was assessed with IV 370 MBq of [^{15}O]water during a 5 minute dynamic PET study acquired using 5 sec frame times. The AT_1R was imaged during a second, 95 min long dynamic PET study, when 391 ± 20 MBq AT_1R specific [^{11}C]L-159,884 was injected. The early PET images obtained 0-2 minutes post injection of [^{11}C]L-159,884 also showed decreased radioligand accumulation, while the delayed images integrated over 55 – 95 minutes showed higher retained activity in the stenotic kidney compared to the contralateral kidney.

The average blood pressure values were significantly increased in the four animals that developed RVH compared to the controls (Mann-Whitney, $p<0.0001$). The circulating PRA and Ang II were increased in these animals, but the difference was not statistically significant. After the last PET study was finished, the animals were euthanized for tissue harvesting. *In vitro* binding assays of isolated glomeruli demonstrated a trend towards increased AT_1R density (Bmax), although the difference was not statistically significant.

In vivo binding parameters demonstrated higher AT_1R binding in the hypoperfused kidney compared to the controls. Radioligand retention was significantly higher in the hypoperfused kidney (0.054) than in controls (0.032; $p<0.0001$; Mann–

Whitney), which resulted in a significant increase in the L/R ratio of radioligand retention (from 0.92 to 1.71; $p<0.0001$, Mann–Whitney). Patlak's Ki was increased in the hypoperfused kidney (from 0.018 to 0.025), and the L/R ratio of Ki was increased also (from 0.93 to 1.35), but these changes did not reach statistically significant levels. The distribution volume (DV) was increased in the hypoperfused kidney (from 4.41 to 7.90), but only the L/R ratio of distribution volumes in the RVH dogs was significantly higher (RVH: 1.936 vs. control 0.933; $p<0.0001$; Mann-Whitney). The AT_1R binding and density correlated positively with blood pressure changes in the RVH animals and negatively with the perfusion of the hypoperfused kidney (Spearman).

CONCLUSIONS

1. AT_1R binding characteristics and PET imaging of [^{11}C]KR31173

- Biodistribution and pharmacological studies in mice showed high activity and specific binding of the original and improved [^{11}C]KR31173 radioligand in mouse adrenals and kidneys.
- The MK-996, KR21173 and SK-1080 significantly blocked the uptake of [^{11}C]KR31173 in the adrenals, lungs, heart and kidneys.
- Pretreatment with AT_2 antagonist PD123,319 resulted in no statistically significant change of binding in the heart, lungs, kidneys, adrenals, indicating the high selectivity of [^{11}C]KR31173.
- The small animal PET studies of mouse kidneys confirmed the results of *ex vivo* biodistribution and pharmacological studies.
- In clinical PET studies the [^{11}C]KR31173 showed high uptake in the renal cortex of dogs, and it was significantly blocked after pretreatment by the AT_1R antagonist SK-1080.
- In clinical PET studies [^{11}C]KR31173 showed significantly higher uptake in the renal cortex of the baboon than [^{11}C]L-159-884, and showed significantly higher inhibition after the pretreatment by the AT_1R antagonist SK-1080.
- In dogs the observed metabolism of [^{11}C]KR31173 significantly decreased with the new radiosynthesis method, and its serum protein binding was lower than [^{11}C]L-159,885 in dog, baboon and human plasma.
- As previous studies showed, [^{11}C]L-159-884 is an excellent radioligand for PET studies in dogs. [^{11}C]KR31173 has higher uptake and specific binding in primates, which indicates that this tracer has the potential to be used in human PET studies.

2. *Investigation of the effects of acute and chronic angiotensin converting enzyme inhibitor (ACEI) treatment on the AT_1R density in the dog kidney*

- Our work was the first study in which the effect of ACEI on AT_1R binding of the kidney is determined *in vivo* with PET imaging.
- After chronic ACEI treatment, under conditions in which the level of circulating angiotensin II was unchanged, all AT_1R binding parameters – Patlak's K_i, radioligand retention and tissue activity was significantly increased in the dog renal cortex.
- After acute ACEI treatment the radioligand binding parameters were increased, but only the change of radioligand retention was significantly increased.
- In the glomeruli of the chronic ACEI-treated animals significantly higher *Bmax* values were measured *in vitro*, which together with the unchanged *Kd* values, confirms our *in vivo* data.

3. *Investigation of the effects of experimental renovascular hypertension on the AT_1R density in the dog kidney*

- Our work was the first study in which the effect of Goldblatt-type 2K,1C RVH on AT_1R binding of the kidney was determined *in vivo* with PET imaging. High AT_1R binding was measured in the hypoperfused kidney, which was confirmed by *in vitro* studies.
- The increase of AT_1R binding and density closely correlated with reduced kidney perfusion and elevated arterial blood pressure.
- If confirmed in humans, these experiments could make the AT_1R an attractive and valuable molecular imaging target to improve the diagnosis of RVH, and to verify the efficacy of revascularization.

ORIGINAL PAPERS

Publications related to the dissertation

Zober TG, Fabucci ME, Zheng W, Brown PR, Seckin E, Mathews WB, Sandberg K, Szabo Z. (2008) Chronic ACE inhibitor treatment increases angiotensin type 1 receptor binding in vivo in the dog kidney. Eur J Nucl Med Mol Imaging 35: 1109-16. (IF: 2.121)

Zober TG, Mathews WB, Seckin E, Yoo SE, Hilton J, Xia J, Sandberg K, Ravert HT, Dannals RF, Szabo Z. (2006) PET Imaging of the AT1 receptor with [11C]KR31173. Nucl Med Biol 33: 5-13. (IF: 4.101)

Mathews WB, Yoo SE, Lee SH, Scheffel U, Rauseo PA, Zober TG, Gocco G, Sandberg K, Ravert HT, Dannals RF, Szabo Z. (2004) A novel radioligand for imaging the AT1 angiotensin receptor with PET. Nucl Med Biol 31: 571-4. (IF: 2.173)

Abstracts related to the dissertation

Zober TG, Brown RP, Sandberg K, Hilton J, Bluemke DA, Sy MA, Rauseo P, Mathews WB, Ravert HT, Dannals RF, Szabo Z. (2003) PET demonstrates upregulation of the renal angiotensin-II (AT1) receptors in experimental renovascular hypertension. J. Nucl. Med. 44: 142P-142P.

Publications not related to the dissertation:

Mathews WB, Zober TG, Ravert HT, Scheffel U, Hilton J, Sleep D, Dannals RF, Szabo Z. (2006) Synthesis and in vivo evaluation of a PET radioligand for imaging the endothelin-A receptor. Nucl Med Biol 33: 15-9. (IF:2.121)

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