Sigma-1 Receptor Imaging in the Brain
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Small-animal PET with a sigma ligand, $^{11}$C-SA4503, detects spontaneous pituitary tumors in aged rats

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ABSTRACT

Pituitary tumors are often detected only post-mortem or at late-stages of the disease when they are macroadenomas with a low surgical cure rate. Spontaneous pituitary tumors occur in rats over one year of age. In an ongoing study of changes in sigma-1 agonist binding related to ageing, several of our rats developed such tumors. The aim of the current study was to assess the kinetics of $^{11}$C-SA4503 in tumor and brain, and to evaluate the utility of this tracer in the detection of pituitary tumors.

Methods: MicroPET scans of the brain region of male Wistar Hannover rats (age 18 to 32 months) were acquired using the sigma-1 agonist tracer $^{11}$C-SA4503. The time-dependent uptake of $^{11}$C in the entire brain, tumor or normal pituitary, and thyroid was measured. Two-tissue compartment model was fitted to the PET data, using metabolite-corrected plasma radioactivity as input function.

Results: Pituitary tumors showed up as bright hot-spots in the scans. The total distribution volume ($V_T$) of the tracer in tumor was significantly higher than in the normal pituitary. Surprisingly, a higher $V_T$ was also seen in the brain and thyroid tissue of animals with pituitary tumors compared to healthy rats. The increase in $V_T$ in brain and thyroid was not related to a change of non-displaceable binding potential ($BP_{ND}$), but rather, to an increase of the partition coefficient ($K_1/k_2$) of $^{11}$C-SA4503. The increase in $V_T$ in the tumor on the other hand was accompanied by a significant increase in $BP_{ND}$. Western blotting analysis indicated that pituitary tumors over expressed sigma-1 receptors.

Conclusion: The over expression of sigma-1 receptors in the spontaneous pituitary tumors is detected as an increase in the uptake and $BP_{ND}$ of $^{11}$C-SA4503. Therefore, this tracer may have promise for the detection of pituitary adenomas, using PET.

Keywords: sigma receptor, $^{11}$C-SA4503, spontaneous pituitary tumor, kinetic analysis, microPET.
INTRODUCTION

Pituitary tumors (adenoma of pituitary gland) are among the five most common intracranial neoplasms (1, 2). Pituitary adenomas grow slowly and metastasize rarely (3). Despite their benign nature, they can cause significant morbidity (4). For example, pituitary tumors can lead to Cushing syndrome, pituitary hyperthyroidism, acromegaly, Nelson syndrome or impotence (5, 6). Unfortunately, pituitary tumors are very difficult to diagnose and often remain undetected until post-mortem examination or during late-stage disease (7, 8). At late stages, the majority of pituitary tumors will be macroadenomas with a low surgical cure rate (3). Diagnostic problems arise from the fact that they can manifest themselves by varying symptoms. Functioning tumors hypersecrete various hormones (e.g. prolactin, thyroid-stimulating hormone, and growth hormone), and non-functioning tumors (non-secreting) grow usually undetected until they compress surrounding structures (e.g. optic nerves) or prevent normal functioning of the pituitary gland (9). In addition, large pituitary tumors can cause occasionally fatal damage to the brain due to a sustained increase in the intracranial pressure (10).

Because hormonal or symptom examination gives a rather poor differential diagnosis, current diagnostic methods include imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI). CT and MRI imaging allows avoidance of biopsies and significantly improves pituitary tumor localization (9, 11, 12). However, CT and MRI cannot distinguish between tumor and scar tissue or monitor biochemical and functional aspects of the tumor.

There is no tracer of first choice for pituitary tumor imaging using nuclear medicine (4), although such lesions have been visualized e.g. with \(^{18}\)F-fluorodeoxyglucose (\(^{18}\)F-FDG), radioactively labeled somatostatin analogs, such as \(^{111}\)In-pentetreotide, and dopamine \(D_2\) receptor-targeting radiopharmaceuticals, such as \(^{18}\)F-fluoroethyl-spiperone (\(^{18}\)F-FESP) (1, 4, 7). The widespread use of \(^{18}\)F-FDG in positron emission tomography (PET) imaging in the last two decades increased the detection of incidentalomas of the pituitary gland (7, 13). However, pituitary tumors are slow growing and highly differentiated, which makes \(^{18}\)F-FDG unsuitable for their imaging (4). Furthermore, \(^{18}\)F-FDG poorly distinguishes between non-/neoplastic lesion, benign/malignant lesions or inflammation, and accumulation of this tracer in pituitary gives an ambiguous interpretation (7, 13, 14, 15). Huyn et al. showed that only 40.8% the of patients with focal pituitary \(^{18}\)F-FDG accumulation presented with pathologic lesions (7). On the other hand, somatostatin receptor and dopamine \(D_2\) receptor-targeting radiopharmaceuticals have only limited clinical usefulness, because expression of these receptors is dependent on the hormones that the pituitary tumor secretes (e.g. higher expression of somatostatin receptors in tumors producing growth hormone/thyroid-stimulating hormone, or higher expression of dopamine \(D_2\) receptors in non-secreutive tumors) (4, 12, 16).

Tumor cells frequently overexpress sigma receptors. Sigma receptors are unique transmembrane proteins, classified into two subtypes, sigma-1 and
Sigma-1 receptors were studied in more detail than sigma-2 receptors since the sigma-1 but not the sigma-2 gene has been sequenced and cloned (18). Sigma-1 receptors were shown to be an intraorganelle signaling and cell survival modulator at the endoplasmic reticulum-mitochondria junctions (19). The sigma-2 subtype was recently identified as the progesterone receptor membrane component 1 (20). Sigma-1 receptors are expressed at rather high densities in the brain and pituitary gland (21). However, the presence of sigma-1 receptors in pituitary tumors has not yet been examined.

$^{11}$C-SA4503 ($^{11}$C-labeled 1-[2-(3,4-dimethoxyphenethyl]-4-(3-phenylpropyl)piperazine dihydrochloride) is a ligand with high affinity for sigma-1 ($IC_{50}$ value 17.4 nM) and lower affinity for sigma-2 receptors (1784 nM), and commonly employed for PET(17). We have previously reported that microPET with $^{11}$C-SA4503 shows high tracer accumulation in the mammalian brain (22). $^{11}$C-SA4503 was already tested in healthy volunteers, and in patients with Alzheimer’s disease (23) or Parkinson’s disease (24). Furthermore, microPET with $^{11}$C-SA4503 in rats successfully detected subcutaneously-grown C6 gliomas, had 10-fold C6 tumor selectivity over a turpentine-induced sterile inflammation, and showed an early decrease in tumor uptake following systemic doxorubicin treatment which corresponded to a loss of sigma-1 receptors from the tumors (25, 26).

In the current study, we examined if spontaneously developed pituitary tumors in aged rats can be detected and distinguished from the normal pituitary by microPET imaging with $^{11}$C-SA4503. Also, we performed kinetic analysis of tracer uptake in the brain, pituitary tumors and thyroid gland, using a 2-tissue compartment model (2-TCM) fit to assess changes in partition coefficient ($K_{1}/k_{2}$), non-displaceable binding potential ($BP_{ND}$), and total distribution volume ($V_{T}$). Finally, the impact of a pituitary tumor on uptake of $^{11}$C-SA4503 in peripheral organs was examined, since a pituitary tumor may affect the vascular properties and perfusion of other tissues because of altered hormone levels.

**MATERIALS AND METHODS**

$^{11}$C-SA4503 Synthesis

The radioligand 1-[2-(3,4-dimethoxyphenethyl]-4-(3-phenylpropyl)piperazine ($^{11}$C-SA4503) was prepared by reaction of $^{11}$C-methyl iodide with 1-[2-(4-hydroxy-3-methoxy-pentethyl]-4-(3-phenylpropyl)piperazine dihydrochloride (4-O-demethyl SA4503), according to a published method (27). The decay corrected radiochemical yield was ~ 24%, the specific radioactivity was > 100 TBq/mmol at the moment of injection and radiochemical purity > 98%. The $^{11}$C-SA4503 solution had a pH of 6.0 to 7.0.
Animal Model

Experiments were performed in male Wistar Hannover rats (HsdHan™:WIST) aged 18 to 32 months. Animals were either purchased from Harlan (Boxmeer, The Netherlands) or acquired from Semmelweis University. It was previously reported that more than 37% of male Wistar Hannover rats above 1-year of age may develop pituitary tumors (28). Additional pituitary tumors and normal pituitary for western blotting were obtained from rats of similar ages from Semmelweis University. The rats were housed in Macrolon cages on a layer of wood shavings in a room with constant temperature (21 ± 2°C) and fixed 12-hour light-dark regime (light phase from 7:00 to 19:00 hours). Food (standard laboratory chow, RMH-B, Hope Farms, The Netherlands) and water were available ad libitum. After arrival the rats were acclimatized for at least seven days. Experiments were performed by licensed investigators in compliance with the Law on Animal Experiments in The Netherlands. The protocol was approved by the Committee on Animal Ethics of the University of Groningen. Animals were assigned to the healthy control (n = 8) or tumor-bearing group (n = 5) after autopsy.

Arterial Blood Sampling

Before microPET scanning, an arterial cannula was placed in each rat for blood sampling and determination of the time course of radioactivity in plasma. For this purpose, rats were anesthetized with isoflurane in medicinal air (5% for induction 2% for maintenance). An incision was made parallel to the femoral artery. The femoral artery was separated from the femoral vein and temporarily ligated to prevent leakage of blood. A small incision was made in the artery and a cannula was inserted (0.8 mm outer, 0.4 mm inner diameter). The cannula was secured to the artery with a suture and attached to a syringe filled with heparinized saline. From each rat, fifteen arterial blood samples (volume 0.1 to 0.15 ml) were collected at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2.0, 3, 5, 7.5, 10, 15, 30, 60 and 90 minutes after 11C-SA4503 injection and start of the microPET scan. Plasma was obtained from the blood by centrifugation (5 min in Eppendorf-type centrifuge at 13,000 rpm). Radioactivity in plasma samples was determined using a calibrated gamma counter (CompuGamma CS 1282, LKB-Wallac, Turku, Finland). Plasma data was corrected for radioactive metabolites based on the age of the rat as below.

In a separate group of animals, larger volumes of blood ranging from 0.4 to 1.6 ml was collected at 5, 10, 20, 40 and 60 min and a metabolite analysis was performed using a method similar to one previously published for 11C-SA4503 (29). Briefly, plasma was obtained by centrifugation (2 min in Eppendorf-type centrifuge at 13,000 rpm) and de-proteinized using one third the volume of 20% trichloroacetic acid in acetonitrile. The mixture was centrifuged for 2 min at 13,000 rpm and the supernatant injected in a reversed-phase HPLC system to separate the parent tracer and metabolites. A MicroBondapak C18 (7.8 x 300 mm) column and a mobile phase consisting of a mixture of acetonitrile and 50
mM sodium acetate buffer (pH 7.2; 1/1, v/v) at a flow rate of 3 mL/min were used. The eluate was collected in 30 sec fractions for 15 min and radioactivity in the samples was measured using a gamma counter.

**MicroPET Scan**

Two rats were scanned simultaneously in each scan session, using a Siemens/Concorde microPET camera (Focus 220). They were positioned in the camera in transaxial position with their heads and neck in the field of view. First, a transmission scan of 515 seconds with a Co-57 point source was obtained for attenuation and scatter correction of 511 keV photons by tissue. Subsequently, the first rat was injected through the penile vein with $^{11}$C-SA4503 (31±16 MBq, volume < 1 ml). The emission scan was started with tracer injection of the first rat; whereas the second animal was injected a few minutes later. A list-mode protocol was used with 90 min acquisition time (analysis performed for first 74 min from tracer injection). Reconstructions were performed using microPET Manager 2.3.3.6 (Siemens). The list-mode data of the emission scans were reframed into a dynamic sequence of 8x30s, 3x60s, 2x120s, 2x180s, 3x300s, 3x600s, 1x720s, 1x960s frames. The data were reconstructed per time frame employing an iterative reconstruction algorithm (OSEM2D with Fourier rebinning, 4 iterations and 16 subsets). The final datasets consisted of 95 slices with a slice thickness of 0.8 mm, and an in-plane image matrix of 128 x 128 pixels. Voxel size was 0.5 x 0.5 x 0.8 mm. The linear resolution at the center of the field-of-view was about 1.5 mm. Data sets were fully corrected for decay, random coincidences, scatter and attenuation.

**MicroPET Data Analysis**

The images obtained from the scan were co-registered with an MRI template (30) for drawing the three-dimensional regions of interest (ROIs) over the whole brain and healthy pituitary. ROIs were also drawn over the thyroid and pituitary tumor using Inveon Research Workplace software (Siemens). Time-activity curves (TACs) were calculated for each of these regions. Tracer uptake was expressed as a PET-SUV, assuming a specific gravity of 1 g/cm³ for brain tissue and blood plasma. The parameter SUV is defined as: $\text{SUV} = \frac{\text{tissue activity concentration (MBq/g)} \times \text{animal body weight (g)}}{\text{injected dose (MBq)}}$.

Kinetic analysis was performed by fitting a standard four-parameter, two-tissue compartment model (2-TCM) to the dynamic PET data, using metabolite-corrected plasma radioactivity from arterial blood samples as input function. The 2-TCM is described in (31); its parameters are rate constant for transport from arterial plasma to tissue ($K_1$), tissue to arterial plasma ($k_2$), free to bound compartment in tissue ($k_3$) and bound to free compartment in tissue ($k_4$). Where plasma data was not available, a population average corrected for injected dose and weight of individual animal was used as input. Software routines for MatLab 7 (The MathWorks, Natick, MA), written by Dr. A.T.M. Willemsen (University
Medical Center Groningen), were used for curve fitting. The blood volume was fixed at 0.036 and the rate constants $K_1$, $k_2$, $k_3$, and $k_4$ estimated from the curve fit. Partition coefficient was calculated as $K_1/k_2$, $BP_{ND}$ was calculated as $k_3/k_4$ and $V_T$ was calculated as $K_1/k_2$ * (1 + $k_3/k_4$).

**Ex vivo Biodistribution**

After the scanning period, animals were terminated by extirpation of the heart. Blood was immediately collected, and plasma and a cell fraction were separated by short centrifugation (5 min at 13,000 rpm). Several tissues (see Tables 1 and 2) were excised. All tissue samples were weighed. Radioactivity in tissue samples and in a sample of the injected tracer solution (infusate) was measured using a gamma counter with automatic decay correction. The data are presented as SUV. Tissue-to-plasma and pituitary or tumor-to-brain concentration ratios of radioactivity were calculated.

**Western Blotting**

A portion of the pituitary tumors (n=5) and the entire pituitary of control animals (n=5) were homogenized in lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl pH 8.0, 2% Nonidet P-40, 10% glycerol and protease inhibitors. Total protein was used for analysis rather than a plasma membrane fraction, since most sigma-1 receptors are known to be intracellular and the intracellular receptors are functionally important (17, 19, 21). Moreover, our ligand $[^{11}C]SA4503$ visualizes the entire sigma-1 receptor population because of its lipophilicity. The homogenate was sonicated for 30s in a cold pack. Lysates were centrifuged for 15 min at 15300g at 4°C. Supernatants were collected and stored at -20°C until use. The concentration of protein was determined with a Bradford assay. 20 µg of protein were electrophoresed on 8-12% (v/v) polyacrylamide SDS-PAGE gels. Proteins were electrotransferred onto PVDF membranes (Amersham, Piscataway, NJ). The nonspecific binding of immunoproteins was blocked with 5% non-fat dry milk for 2 hours at RT. After blocking, the membrane was incubated with primary antibodies overnight at 4°C. The primary antibody recognizing sigma-1 receptors was ab89655, an anti-Sig-1R antibody purchased from AbCam. The membrane was rinsed in TBS containing 0.1% Tween 20 (TBS-T) followed by 1h incubation with HRP-conjugated secondary antibody at room temperature. After incubation, the membranes were repeatedly washed in TBS-T and incubated with an enhanced chemiluminescence reagent (ECL plus, RPN 2132, Amersham). The protein bands were visualized on X-ray films. The optical density of the protein bands were quantified using ImageJ (National Institutes of Health, Bethesda, MD), and standardized to β-actin (1:2000, sc-47778 Santa Cruz).

**Statistics**

All results are expressed as mean ± SEM. Differences between groups were examined by unpaired two-tailed t-test or 2-way ANOVA, followed by a post hoc
Bonferroni test, where applicable. A *P* value < 0.05 was considered statistically significant. Correlations were assessed using Pearson correlation coefficient (r) and considered strong when *r*² was at least 0.7 and significant when *P* value < 0.05.

**RESULTS**

**Tumor Visualization on PET Images**

Spontaneous pituitary tumors developed in 38% of the aged rats. These tumors were clearly visible in the \(^{11}\)C-SA4503 scans (**Figure 1**). The brains of animals with pituitary tumors also appeared to take up more tracer than normal brain. The thyroid glands were in the field of view and they appeared to take up more of the tracer in the tumor-bearing rats than in the normal aged rats (**Figure 1**).

**Figure 1.** \(^{11}\)C-SA4503 microPET images of: A) a healthy aged rat; B) an aged rat with spontaneous pituitary tumor weighing 17 mg. TRANS = transverse, COR = coronal, SAG = sagittal view. Solid arrows indicate the healthy pituitary or pituitary tumor, dashed arrow position of the thyroid gland.
Kinetics of Radioactivity in Plasma and Brain

Metabolite analysis revealed that about 50% of the parent tracer remained intact at 60 minutes, but there was no significant difference between the groups (data not shown). Kinetics of radioactivity in the plasma (metabolite corrected, n=4 each) after a bolus injection of $^{11}$C-SA4503 is shown in Figure 2A. Values from four normal rats and one rat with tumor were not included in this analysis due to nonavailability of plasma samples (failure of the cannula and changed kinetics due to a small depot during tracer injection). A rapid biexponential clearance was observed in both groups. The presence of the pituitary tumor did not significantly affect plasma kinetics of the tracer. Area under the curve (AUC) for normal rats: 18.0 ± 3.1; rats with tumor: 13.1 ± 0.8, ($P = 0.1720$).

Kinetics of $^{11}$C-SA4503 in the pituitary and pituitary tumor, the brain and the thyroid are presented in Figures 3B-D. Tracer uptake was rapid in normal brain, normal pituitary and brain with tumor, with the peak appearing within 5 min of

![Figure 2](image-url). Time-activity curves of $^{11}$C-SA4503 in: A) plasma of normal (n = 5) and tumor-bearing (n = 4) rats; B) normal pituitary (n = 7) and pituitary tumors (n = 5); C) brain of normal (n = 7) and tumor-bearing (n = 5) rats; D) thyroid of normal (n = 7) and tumor-bearing rats (n = 5). Data are expressed as mean ± SEM, differences in the area under the curve (AUC) were tested with unpaired two-tailed t-test.
tracer injection, and was followed by a slow washout. The uptake in the pituitary tumor, while also rapid, reached a plateau within 10 min and no appreciable washout was seen within the scan duration. Uptake of the tracer was significantly higher in the tumor than in the normal pituitary and was about 3-fold higher by the end of the scan (AUC 439.6 ± 104.4 vs 166.6 ± 8.6, \( P < 0.05 \)). Uptake in the tumor was also higher than in the brain of tumor-bearing rats and was about 2-fold higher by the end of the scan. The uptake in the brain of tumor-bearing rats was about 50% higher than in the brain of normal aged rats.

**Kinetic Analysis**

Using the metabolite-corrected arterial plasma as input, 2-TCM was fitted to ROIs drawn around whole brain, normal pituitary, pituitary tumor and thyroid. Where plasma data was not available, a population average corrected for injected dose and weight of individual animal was used as input. The \( V_T \) (Figure 3A) of the tracer was significantly higher \( (P < 0.05) \) in tumor \( (105.0 ± 47.6) \) than in the normal pituitary \( (11.6 ± 1.0) \). Furthermore, the \( V_T \) in the brain tissue of animals with pituitary tumors was higher than in brain tissue of normal rats \( (25.5 ± 3.3 vs 11.8 ± 1.2, P < 0.001) \) (Figure 3B). Additionally, the \( V_T \) in the thyroid of rats with pituitary tumors was higher than in the thyroid of normal rats \( (39.3 ± 5.5 vs 23.1 ± 1.9, P < 0.01) \).

The higher \( V_T \) in the tumor was associated with a significantly higher \( BP_{ND} \) \( (10.7 ± 1.7 vs 4.6 ± 0.5, P < 0.01) \) as well as \( K_f/k_2 \) \( (10.5 ± 4.5 vs 2.2 ± 0.3, P < 0.05) \) (Figures 4A). On the other hand, the higher \( V_T \) in the brain was not related to a change in \( BP_{ND} \), but was due to a higher partition coefficient \( (K_f/k_2) \) of \( ^{11}\text{C}-\text{SA4503} \) \( (6.6 ± 1.3 vs 2.4 ± 0.4, P < 0.01) \) (Figure 3B). Similarly, the higher \( V_T \) in the thyroid was related to a higher \( K_f/k_2 \) \( (8.8 ± 1.3 vs 5.1 ± 0.8, P < 0.05) \), rather than to \( BP_{ND} \). Changes of \( V_T \) and \( K_f/k_2 \) in brain and thyroid were strongly correlated \( (R^2 = 0.81, P < 0.0001, and R^2 = 0.85, P < 0.0001, respectively) \). The correlation between changes in \( BP_{ND} \) was not as strong, but still quite significant \( (R^2 = 0.66, P = 0.0014) \) (Figures 4C). However, changes of \( K_f/k_2 \) in brain and pituitary or tumor correlated only weakly \( (R^2 = 0.38, P < 0.05) \) and changes of \( V_T \) and \( BP_{ND} \) were not correlated at all \( (R^2 = 0.19, P = 0.1372 and R^2 = 0.02, P = 0.6622, respectively) \).

**Biodistribution**

The mass of a normal pituitary was \( 12.7 ± 3.3 \text{ mg} \) (mean ± SD, \( n = 5 \), range 8.6 to 17.7 mg). Spontaneous pituitary tumors weighed \( 70.5 ± 93.1 \text{ mg} \) (mean ± SD, \( n = 8 \), range 14.0 to 274.7 mg). Biodistribution data of \( ^{11}\text{C}-\text{SA4503} \), 90 min after injection, showed a considerable uptake in the pituitary tumor \( (SUV 10.3 ± 2.1) \), which was about 3-fold higher than in the normal pituitary gland. Accumulation of \( ^{11}\text{C}-\text{SA4503} \) in tumor tissue was about 100-fold higher than in plasma and about 6-fold higher than in the adjacent brain (Table 1).

Biodistribution data for the brain areas and peripheral organs are listed in Table 2. The presence of the pituitary tumor was associated with a significantly
higher SUV and tissue-to-plasma ratio of the tracer in all brain areas. SUV values in the peripheral organs of normal and tumor-bearing rats were not significantly different, but tissue-to-plasma ratios in brain, small intestine, liver and spleen of tumor-bearing animals were significantly higher.

**Figure 3.** Kinetic modeling parameters of $^{11}$C-SA4503 in: A) normal pituitary (n = 8) and pituitary tumor (n = 5); B) brain of normal (n = 8) and tumor bearing (n = 5) rats; C). Correlation of $^{11}$C-SA4503 kinetic parameters ($V_T$, $BP_{ND}$, $K_1/k_2$) in the thyroid and brain. Data are expressed as mean ± SEM and were tested with unpaired, two-tailed t-test.
Table 1. Biodistribution of $^{11}$C-SA4503 in the pituitary and tumor

<table>
<thead>
<tr>
<th></th>
<th>Normal Pituitary (n=5)</th>
<th>Pituitary tumor (n=6)</th>
<th>$P$#</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUV</td>
<td>3.28 ± 0.47</td>
<td>10.28 ± 2.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tissue to Brain Ratio</td>
<td>2.90 ± 0.61</td>
<td>5.70 ± 0.77</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tissue to Plasma Ratio</td>
<td>17.29 ± 1.72</td>
<td>101.1 ± 19.42</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Two rats which had large necrotic tumors were not included in this analysis. Data are expressed as mean ± SEM.

**Western Blotting**

Western blotting was performed to quantify the sigma-1 receptor proteins in the pituitary tumor and normal pituitary. Immunoreactive bands for sigma-1 receptor proteins were visualized at 25kDa. The sigma-1 receptor protein to β-actin ratio was significantly higher in the pituitary tumors compared to normal pituitary (1.94 ± 0.21 vs 0.97 ± 0.13, $P < 0.01$) (Figure 4).

**DISCUSSION**

This study demonstrates that spontaneous pituitary tumors in aged rats can be detected and distinguished from normal pituitary and brain by microPET imaging with the sigma-1 ligand, $^{11}$C-SA4503. To the best of our knowledge, this is the first report identifying sigma-1 receptors in pituitary tumors.

For clear visualization of tumor lesions, the ratio of tracer uptake in tumor versus surrounding tissue must be >> 1. In the biodistribution experiments, we
Table 2. Biodistribution of $^{11}$C-SA4503 in the brain and peripheral regions

<table>
<thead>
<tr>
<th>Tissue</th>
<th>SUV Normal Aged (n=7)</th>
<th>SUV With Tumor (n=5)</th>
<th>P#</th>
<th>SUV Normal Aged (n=7)</th>
<th>SUV With Tumor (n=5)</th>
<th>P#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>1.21 ± 0.12</td>
<td>2.08 ± 0.25</td>
<td>&lt;0.001</td>
<td>7.06 ± 1.01</td>
<td>20.67 ± 1.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>1.55 ± 0.05</td>
<td>2.31 ± 0.16</td>
<td>&lt;0.01</td>
<td>8.97 ± 0.79</td>
<td>23.30 ± 2.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rest brain</td>
<td>1.28 ± 0.05</td>
<td>1.87 ± 0.18</td>
<td>&lt;0.05</td>
<td>7.41 ± 0.70</td>
<td>18.54 ± 1.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.32 ± 0.10</td>
<td>0.38 ± 0.19</td>
<td>ns</td>
<td>1.91 ± 0.65</td>
<td>3.59 ± 1.74</td>
<td>ns</td>
</tr>
<tr>
<td>Bladder</td>
<td>3.09 ± 1.29</td>
<td>2.28 ± 0.93</td>
<td>ns</td>
<td>19.81 ± 9.26</td>
<td>22.82 ± 8.62</td>
<td>ns</td>
</tr>
<tr>
<td>Bone</td>
<td>0.43 ± 0.08</td>
<td>0.62 ± 0.08</td>
<td>ns</td>
<td>2.48 ± 0.55</td>
<td>6.45 ± 1.20</td>
<td>ns</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>3.55 ± 1.10</td>
<td>2.72 ± 0.90</td>
<td>ns</td>
<td>18.77 ± 5.09</td>
<td>27.10 ± 8.72</td>
<td>ns</td>
</tr>
<tr>
<td>Heart</td>
<td>0.61 ± 0.05</td>
<td>0.93 ± 0.05</td>
<td>ns</td>
<td>3.49 ± 0.37</td>
<td>9.43 ± 1.00</td>
<td>ns</td>
</tr>
<tr>
<td>Large intestine</td>
<td>2.41 ± 0.27</td>
<td>2.94 ± 0.15</td>
<td>ns</td>
<td>14.30 ± 2.35</td>
<td>30.74 ± 5.18</td>
<td>ns</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3.50 ± 0.38</td>
<td>5.41 ± 0.52</td>
<td>ns</td>
<td>20.26 ± 3.07</td>
<td>55.59 ± 8.43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.50 ± 0.25</td>
<td>4.08 ± 0.55</td>
<td>ns</td>
<td>20.40 ± 2.34</td>
<td>41.62 ± 6.77</td>
<td>ns</td>
</tr>
<tr>
<td>Liver</td>
<td>11.05 ± 0.63</td>
<td>11.68 ± 1.15</td>
<td>ns</td>
<td>63.83 ± 5.83</td>
<td>115.42 ± 6.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung</td>
<td>2.21 ± 0.14</td>
<td>3.85 ± 0.60</td>
<td>ns</td>
<td>12.71 ± 1.18</td>
<td>39.32 ± 6.93</td>
<td>ns</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.30 ± 0.06</td>
<td>0.47 ± 0.08</td>
<td>ns</td>
<td>1.70 ± 0.32</td>
<td>4.83 ± 0.92</td>
<td>ns</td>
</tr>
<tr>
<td>Pancreas</td>
<td>5.74 ± 1.01</td>
<td>5.31 ± 0.77</td>
<td>ns</td>
<td>33.23 ± 6.82</td>
<td>55.07 ± 10.28</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.18 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>ns</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
<td>ns</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>0.09 ± 0.02</td>
<td>0.06 ± 0.01</td>
<td>ns</td>
<td>0.50 ± 0.10</td>
<td>0.64 ± 0.06</td>
<td>ns</td>
</tr>
<tr>
<td>Spleen</td>
<td>4.10 ± 0.29</td>
<td>5.77 ± 0.77</td>
<td>ns</td>
<td>24.13 ± 3.13</td>
<td>58.12 ± 7.82</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Submandibular gland</td>
<td>6.67 ± 0.83</td>
<td>4.90 ± 0.97</td>
<td>ns</td>
<td>37.24 ± 3.93</td>
<td>53.76 ± 16.45</td>
<td>ns</td>
</tr>
<tr>
<td>Urine</td>
<td>4.76 ± 2.07</td>
<td>3.676 ± 1.69</td>
<td>ns</td>
<td>31.26 ± 14.76</td>
<td>40.07 ± 18.42</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM
observed an accumulation of $^{11}$C-SA4503 in the pituitary tumors which was about 6-fold higher than in the adjacent brain and about 100-fold higher than in plasma. Therefore, the pituitary tumors were easily visualized with $^{11}$C-SA4503.

Kinetic modeling indicated that $V_T$ in pituitary tumors increased (by 10-fold) compared to the normal pituitary. These changes in $V_T$ were related to increases in both $K_1/k_2$ and $BP_{ND}$ (4.9-fold and 2.3-fold, respectively). Tracer $V_T$ in the brain of pituitary tumor-bearing rats was increased (by 2.4-fold) compared to brain of healthy rats, due to an increase in $K_1/k_2$ (by 2.7-fold), whereas $BP_{ND}$ was unaltered (Figure 3A). Since pituitary is adjacent to the brain, partial volume effect could play a role in the increased $V_T$ in the brains of rats with pituitary tumor. However, the higher SUV in the brains of animals with pituitary tumor in the biodistribution study suggests that this effect is independent of partial volume effects. A 1.7-fold increase of $K_1/k_2$ (but not $BP_{ND}$) was also observed in the thyroid gland of tumor-bearing rats (Figure 3B).

Increases in the partition coefficient ($K_1/k_2$) of the tracer were observed not only in pituitary tumors, but also in the brain and thyroid. Increases in $K_1/k_2$ are thus not limited to tumor tissue. Pituitary tumors are known to exert global effects on blood flow (32) and different effects on regional blood pressure (32). Altered tissue-to-plasma ratios of $^{11}$C-SA4503 in the brain, small intestine, liver and spleen of tumor-bearing animals (Table 2) may be related to such phenomena, although additional data on hormone levels and tissue perfusion are required to prove the underlying mechanism.

A change in $BP_{ND}$ could indicate a change in either receptor numbers or affinity. If the affinity of $^{11}$C-SA4503 to sigma-1 receptors remains constant during malignant transformation, as has been reported for nonneural tumors (33), the observed increase of $BP_{ND}$ in tumor tissue should reflect an up-regulation of sigma-1 receptors in pituitary tumors. Such up-regulation would be consistent with the moderate (generally 2-3-fold) overexpression of sigma-1 receptors in other tumor tissues (17, 33, 34). Our results from Western blotting also confirmed a 2-fold over-expression of sigma-1 receptors in pituitary tumors. The reason for up-regulation of sigma-1 receptors in tumor tissue is unclear but it may provide the tumor cells with an additional brake on apoptosis (35). Since $BP_{ND}$ values in the brain and thyroid of tumor-bearing rats were not increased, sigma-1 receptor expression in these organs appears to be not affected by the pituitary tumor.

In this study, we did not find any correlation between $BP_{ND}$ and tumor size (data not shown). Similarly, in a study on 95 breast cancer patients, no correlation was observed between sigma-1 receptor levels and tumor size, histological grade or expression of the proliferation marker, Ki-67, although a positive correlation was reported between sigma-1 receptor levels, hormone receptor positivity (in particular progesterone receptor), Bcl-2 expression and the period of disease-free survival (36). Low ratios (< 1) of pro-survival Bcl-2 and pro-apoptotic Bax expression predominate in non-functioning pituitary tumors and pituitary microadenomas (37).
CONCLUSION

The very high uptake of $^{11}$C-SA4503 in pituitary adenomas indicates that it may be worthwhile to test $^{11}$C-SA4503-PET in a clinical setting. Even very small tumors were clearly detected (lesion mass in Figure 1 was 17 mg). This suggests that $^{11}$C-SA4503 may be applied for the detection of microadenomas. Future studies are needed to answer the question whether $^{11}$C-SA4503-PET can discriminate between symptomatic, hormone-secreting and non-functioning (non-symptomatic, non-secretive) tumors.
REFERENCES


19. Hayashi T, Su TP. Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate ca(2+) signaling and cell survival. *Cell*. 2007;131:596-610.


