Selective 5-HT$_{1A}$ Receptor Ligands for PET; A Comparative Study of $[^{11}\text{C}]$ORG13502 and $[^{11}\text{C}]$WAY100635 in Normal and Adrenalectomized Rats*

Abstract

ORG13502 (6-{4-[4-(2-methoxyphenyl)-1-piperazinyl]butyl}-N-methylbenzoxazolinone, 1) and WAY100635 (N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)cyclohexane carboxamide, 2) are highly potent and selective 5-HT$_{1A}$ receptor ligands. We prepared the $[^{11}\text{C}]$-analogues by methylation with $[^{11}\text{C}]$CH$_3$I of the corresponding phenol piperazino precursors. The specific activities of $[^{11}\text{C}]$ORG13502 and $[^{11}\text{C}]$WAY100635 were $>$300 and 1000 Ci/mmol, respectively, after HPLC purification. Total synthesis times were 45 and 30 min, respectively, and the radiochemical yields were $\sim$60% (from $[^{11}\text{C}]$CH$_3$I and decay-corrected). Tissue distribution studies in male Wistar rats revealed that the regional uptake of $[^{11}\text{C}]$WAY100635 after 60 min, but not of $[^{11}\text{C}]$ORG13502 reflected the known 5-HT$_{1A}$ receptor distribution in the rat brain. Pretreatment with the selective 5-HT$_{1A}$ receptor agonist 8-OH-DPAT resulted in substantial blockade of $[^{11}\text{C}]$WAY100635 uptake in 5-HT$_{1A}$ receptor-rich brain regions (70-78% in raphe nuclei, frontal cortex, septum, hippocampus). Adrenalectomy (ADX, 1 or 6 days), which is known to cause 5-HT$_{1A}$ receptor upregulation in rats, had no significant effect on the uptake of $[^{11}\text{C}]$WAY100635. However, the brain uptake of $^{11}\text{C}$ after 24 h ADX was more sensitive to pretreatment with 8-OH-DPAT than in control animals in all examined brain areas, except for cerebellum.

7.1 Introduction

Central 5-HT$_{1A}$ receptors, existing both as somatodendritic autoreceptors at the raphe nuclei and post-synaptically, have been implicated in the pathogenesis of anxiety and depression.$^{1,2}$ Thus, acutely or chronically administered 5-HT$_{1A}$ receptor agonists all

have therapeutic potency in treating these disorders. When given chronically they may alter the 5-HT$_{1A}$ receptor density in various brain areas.\textsuperscript{3} Definitive conclusions on their mode of action can not be drawn because of the lack of appropriate pharmacological tools. A suitable procedure for visualization and quantification of central (and peripheral) 5-HT$_{1A}$ receptors as may be achieved with positron emission tomography (PET) is of great clinical interest. The radioligands developed and evaluated so far mostly were (partial) agonists which, due to unfavourable in vivo kinetic properties, failed as PET-ligands.

WAY100635 (2; Figure 7.1), a selective and silent 5-HT$_{1A}$ receptor antagonist (IC$_{50}$ value of 1.6 nM),\textsuperscript{4} has successfully been labelled and evaluated as a potential in vivo PET-imaging agent.\textsuperscript{5} Quantification of 5-HT$_{1A}$ receptors can be highly valuable for elucidating their role in the pathogenesis of various diseases such as depression and anxiety disorders.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure7.1.png}
\caption{Chemical structures of ORG13502 and WAY100635}
\end{figure}

ORG13502 (1) is a highly potent and selective 5-HT$_{1A}$ receptor agonist (Ki = 0.25 nM) with low intrinsic activity (I.A. of 0.2) and therefore was considered a candidate ligand for labelling with a positron emitter.\textsuperscript{6} In order to compare ORG13502 and WAY100635, the $^{11}$C-labelled congeners were prepared by methylation with $[^{11}\text{C}]$methyliodide of the corresponding ortho-hydroxyphenylpiperazines (Figure 7.2). The second objective was to study the effect of changed 5-HT$_{1A}$ receptor densities on the biodistribution of 5-HT$_{1A}$ receptor ligands. In principle, brain B$_{\text{max}}$ in rats can be altered by adrenalectomy (ADX), which is known to cause an upregulation of the 5-HT$_{1A}$ receptor subtype.\textsuperscript{7,8} Here we report the synthesis of $[^{11}\text{C}]$ORG13502 and
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$[^{11}C]WAY100635$ and the results of biodistribution studies in rat brain in normal and adrenalectomized animals.

### 7.2 Chemistry

$[^{11}C]CH_3I$ was produced from $[^{11}C]CO_2$ ($^{14}$N (p,$\alpha$) $^{11}$C nuclear reaction with 17 MeV protons) using an Anatech robotic system, yielding 15 GBq $[^{11}C]CH_3I$ with a specific activity of more than 1000 Ci/mmol. According to a modified procedure as described by Elsinga et al,$^9$ the $^{11}$C analogues of ORG13502 and WAY100635 were prepared by methylation with $[^{11}C]$methyl iodide of the corresponding phenols. The specific activities were >300 and >1000 Ci/mmol, respectively. In brief, a mixture of $[^{11}C]CH_3I$, the phenol and potassium-t-butoxide in acetonitrile was heated for 5 min in a cap-sealed tube at 110 °C (Figure 7.2). After purification by reversed-phase HPLC the desired compounds were obtained in a radiochemical yield of about 60% (from $[^{11}C]CH_3I$, corrected for decay).

![Figure 7.2. Radiosyntheses of $[^{11}C]$-o-methoxy-phenylpiperazines](figure)

### 7.3 Pharmacology

Tissue distribution studies. A tail vein was catheterized with the rat under anaesthesia and after recovery the animals were kept under light restraint. The radioligands (100 μCi) was injected via the tail vein. Rats were killed after 60 min after injection. Brains were rapidly removed, nine regions sampled and the radioactivity was measured (expressed as a differential absorption ratio [DAR = (counts per min recovered/g tissue)/(counts per min injected/g body weight)]). Blocking experiments
were performed with 8-OH-DPAT (Table 7.1). If required, animals were adrenalectomized (ADX) 1 or 6 days before the experiments (Table 7.3).

Metabolism of $[^{11}\text{C}]$ORG13502. Blood-samples (200 μL) were taken at different time intervals. After removal of the proteins, the supernatant was injected onto an HPLC-system. HPLC-samples were collected every 30 s and the radioactivity content was determined (Table 7.2).

7.4 Results and Discussion

Chemistry. The methylation reaction of desmethyl ORG13502 and WAY100635 could be performed conveniently in reasonable radiochemical yields of about 60%. Only minor amounts of by-products (probably due to N-alkylation) were observed and the radioligands could be separated easily from the precursors by HPLC. An unexpected difference of about 700 Ci/mmol in specific activities between the radiolabelled products was found in favor of $[^{11}\text{C}]$WAY100635, although the reaction conditions and the position of labelling of both precursors were identical. No obvious explanation can be given for this observation. Advantageous in the synthesis of $[^{11}\text{C}]$WAY100635 was the direct application of the reaction mixture on the semi-preparative column. The use of an ethanol/water mixture as the eluent, instead of methanol/ phosphate buffer, saved an additional evaporation step which had to be performed in the synthesis of $[^{11}\text{C}]$ORG13502. These two ‘short-cuts’ resulted in a 15 min reduction of the total synthesis time of $[^{11}\text{C}]$WAY100635.
Table 7.1. Distribution Studies in Rat Brain after 100 µCi $\left[^{11}C\right]$ORG13502 (1) or $\left[^{11}C\right]$WAY100635 (2) Injection and Pretreatment with 8-OH-DPAT.

<table>
<thead>
<tr>
<th>brain area</th>
<th>$\left[^{11}C\right]$-1</th>
<th>$\left[^{11}C\right]$-1 + 8-OH-DPAT</th>
<th>$\left[^{11}C\right]$-2</th>
<th>$\left[^{11}C\right]$-2 + 8-OH-DPAT</th>
<th>% Reduction of $\left[^{11}C\right]$-2 binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td>1.77±0.15</td>
<td>1.06±0.17*</td>
<td>0.11±0.01</td>
<td>0.12±0.02</td>
<td>-</td>
</tr>
<tr>
<td>Striatum</td>
<td>2.03±0.21</td>
<td>1.31±0.15**</td>
<td>0.22±0.03</td>
<td>0.14±0.02*</td>
<td>36</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.80±0.22</td>
<td>1.21±0.28***</td>
<td>0.29±0.06</td>
<td>0.20±0.09</td>
<td>40</td>
</tr>
<tr>
<td>Med. ol.</td>
<td>2.07±0.40</td>
<td>1.56±0.32***</td>
<td>0.50±0.08</td>
<td>0.20±0.04*</td>
<td>57</td>
</tr>
<tr>
<td>Oc. cortex</td>
<td>2.34±0.40</td>
<td>1.16±0.23***</td>
<td>0.65±0.08</td>
<td>0.23±0.02**</td>
<td>62</td>
</tr>
<tr>
<td>Fr. cortex</td>
<td>1.99±0.21</td>
<td>1.24±0.46***</td>
<td>0.78±0.09</td>
<td>0.20±0.02**</td>
<td>74</td>
</tr>
<tr>
<td>Raphe Nuclei</td>
<td>2.27±0.58</td>
<td>1.56±1.34</td>
<td>0.91±0.15</td>
<td>0.25±0.09*</td>
<td>70</td>
</tr>
<tr>
<td>Septum</td>
<td>1.93±0.22</td>
<td>1.33±0.33*</td>
<td>1.39±0.19</td>
<td>0.37±0.08***</td>
<td>74</td>
</tr>
<tr>
<td>Hipp.</td>
<td>1.73±0.30</td>
<td>1.07±0.25</td>
<td>1.55±0.37</td>
<td>0.16±0.06***</td>
<td>78</td>
</tr>
</tbody>
</table>

The uptakes are expressed as differential absorption ratios (D.A.R.) , 60 min post-injection. Errors are in SEM. P<0.05 is denoted with *, P<0.01 with ** and P<0.005 with *** (Vehicle vs blocked). % of reduction of $\left[^{11}C\right]$WAY100635 after 8-OH-DPAT pretreatment calculated from the brain area/cerebellum ratios (Table 7.3).

Pharmacology. The regional uptake of $\left[^{11}C\right]$WAY100635, but not of $\left[^{11}C\right]$ORG13502, correlated with the known 5-HT$_{1A}$ receptor distribution in the rat brain (Tables 7.1 and 7.3).$^{10}$ The ex vivo data obtained with rat brain membranes showed that brain uptake of $\left[^{11}C\right]$ORG13502, a highly potent and selective 5-HT$_{1A}$ receptor ligand, was homogeneous throughout the brain and partially reduced upon pretreatment with the 5-HT$_{1A}$ agonist 8-OH-DPAT. This reduction was also observed in cerebellum which is a brain area essentially devoid of 5-HT$_{1A}$ receptors.$^{11}$ At first, rapid formation of radioactive metabolites was thought to be the main cause of strong non-specific binding. Preliminary data on rat plasma, however, revealed that $\left[^{11}C\right]$ORG13502 was only slowly metabolized; more than 50% of the parent compound still being present in plasma after 20 min (Table 7.2). Apparently, rapid metabolism is not the cause of the failure of $\left[^{11}C\right]$ORG13502 as a radioligand. Other pharmacokinetic aspects of this compound have not been investigated and may be difficult to tackle. The lipophilicity of ORG13502 (logP value of 3.6* (3.4)#) is comparable with that of WAY100635 (3.3)#. The calculated logD values at pH 7.4 of both compounds were 3.0. All in all, this suggests that the lipophilicity of ORG13502 has no major contribution to the observed non-specific binding.

* Experimentally determined by N. V. Organon.
# Calculated with Pallas 1.2 (CompuDrug Chemistry Ltd. (c) 1994)
It has been speculated that only pure 5-HT$_{1A}$ receptor antagonists are suitable as ligands for PET, since radiolabelled 5-HT$_{1A}$ receptor agonists, such as $[^{11}C]$8-OH-DPAT (3) and $[^{11}C]$OSU191 (4), are thought to compete unsuccessfully with the endogenous neurotransmitter (5-HT) for binding sites. However, contrasting results were disclosed by Thorell and co-workers who presented the 5-HT$_{1A}$ receptor agonist $[^{11}C]$(R)-10-methyl-11-hydroxyaporphine ([$^{11}$C]HYMAP, 5) as a promising PET-ligand, although no biodistribution data were given.

The agonist-receptor interaction seems to be relatively short in case of G-protein-coupled receptors (Figure 7.4). The formation of a ternary complex of an agonist, a receptor and an inactive, GDP-bound, G-protein facilitates the exchange of GDP by GTP. Hereafter, the agonist, receptor and G-protein rapidly dissociate resulting in free receptor subunits having a low affinity state, which can no longer accommodate agonist ligands. In contrast, antagonist radioligands have proven to be efficient through long duration and high affinity binding therefore allowing autoradiographic visualization and quantification of the specific labelling. In chapter 6, ORG13502 was found to be more potent in inducing the agonist effect (EC$_{50}$ of 0.5 nM) than the antagonist effect (IC$_{50}$ of 5 nM). WAY100635 was totally inactive in the agonist assay. This suggests that at the low concentration employed in these PET-studies, ORRG13502 rather may behave as an 5-HT$_{1A}$ receptor agonist. Therefore, it can not be excluded that $[^{11}C]$ORG13502 failed as an in vivo PET-ligand due to its agonist properties and (consequently) the pharmacodynamic properties.
We found similar uptake patterns as Hume et al.\textsuperscript{5a} and Pike et al.\textsuperscript{5b} in rat brain after injection of $[^{11}\text{C}]$WAY100635. At 60 min after injection, the ratio of radioactivity in 5-HT\textsubscript{1A} receptor-rich regions (e.g. septum and hippocampus) to that in cerebellum reached ca. 13 and 15, respectively. Substantial blockade of $^{11}$C uptake was achieved by pretreatment of rats with 8-OH-DPAT (Table 7.1). The higher the 5-HT\textsubscript{1A} receptor density in a particular brain area, the more effective was 8-OH-DPAT in blocking the $[^{11}\text{C}]$WAY100635 uptake. Moderate blockade was observed in areas with low receptor density, such as striatum (36%), whereas a greater reduction of $[^{11}\text{C}]$WAY100635 binding was observed in receptor-rich areas of the brain (78% in hippocampus).

In order to study changes in receptor densities, rats were adrenalectomized (ADX), which is known to cause upregulation of the 5-HT\textsubscript{1A} receptor.\textsuperscript{7,8} These authors found a $\sim$30% increase of hippocampal 5-HT\textsubscript{1A} receptor density after 1–7 days ADX utilizing autoradiography with $[^{3}\text{H}]$8-OH-DPAT and in situ hybridization techniques. Surprisingly, in our experiments, none of the studied brain areas showed an increased uptake of $[^{11}\text{C}]$WAY100635, as compared to normal rats after 1 or 6 days ADX (Table 7.3).
### Table 7.3. Distribution Studies in Rat Brain with $^{11}$CWAY100635.

<table>
<thead>
<tr>
<th>brain area</th>
<th>Vehicle</th>
<th>Normal + 8-OH-DPAT\textsuperscript{a}</th>
<th>ADX (24 h)</th>
<th>ADX (24 h) + 8-OH-DPAT\textsuperscript{b}</th>
<th>ADX (6 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Striatum</td>
<td>2.06±0.21</td>
<td>1.32±0.30</td>
<td>1.96±0.38</td>
<td>0.99±0.14</td>
<td>2.90±0.81</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.50±0.29</td>
<td>1.49±0.35</td>
<td>2.31±0.26</td>
<td>0.80±0.08*</td>
<td>3.16±0.54</td>
</tr>
<tr>
<td>Med. obl.</td>
<td>4.50±0.43</td>
<td>1.93±0.55</td>
<td>5.36±1.15</td>
<td>1.06±0.22*</td>
<td>5.10±0.52</td>
</tr>
<tr>
<td>Oc. cortex</td>
<td>5.92±0.41</td>
<td>2.23±0.44</td>
<td>6.60±0.95</td>
<td>1.27±0.22*</td>
<td>7.00±1.09</td>
</tr>
<tr>
<td>Fr. cortex</td>
<td>7.24±0.59</td>
<td>1.87±0.27*</td>
<td>6.57±0.81</td>
<td>1.44±0.20*</td>
<td>7.28±1.00</td>
</tr>
<tr>
<td>Raphe Nuclei</td>
<td>8.05±0.71</td>
<td>2.42±1.05*</td>
<td>5.78±0.71</td>
<td>1.69±0.38*</td>
<td>6.06±0.54</td>
</tr>
<tr>
<td>Septum</td>
<td>12.91±1.27</td>
<td>3.42±0.90*</td>
<td>10.34±2.11</td>
<td>1.66±0.46*</td>
<td>10.50±1.30</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>14.58±1.13</td>
<td>3.22±0.28*</td>
<td>12.78±1.83</td>
<td>2.59±0.48*</td>
<td>14.79±2.73</td>
</tr>
</tbody>
</table>

The uptakes are expressed as brain area/cerebellum ratios, 60 min post-injection. Errors are in SEM. P<0.05 is denoted with * (\textsuperscript{a} Vehicle vs blocked and \textsuperscript{b} ADX (24 h) vs blocked ADX (24 h)).

Interestingly, the brain uptake of $^{11}$C in animals after 24 hours ADX seemed more sensitive to pretreatment with 8-OH-DPAT than that in control animals in all examined brain regions, except for cerebellum, which suggests a shift from a low affinity state to a high affinity state, rather than an upregulation of the 5-HT\textsubscript{1A} receptor. However, the effect of ADX on brain uptake did not reach statistical significance due to large individual variances. Hume et al.\textsuperscript{5a} checked the ‘specific’ signal of $[^3]$HWAY100635 by pre-dosing the rats with compounds of known selectivity. Pretreatment with 8-OH-DPAT resulted in an average 77% reduction of the specific signal, however, the 8-OH-DPAT-insensitive binding corresponded regionally with both the specific signal and the 8-OH-DPAT-sensitive binding. Additionally, autoradiography studies revealed that $[^3]$HWAY100635 could not discriminate between G-protein-coupled and G-protein-uncoupled 5-HT\textsubscript{1A} receptors.\textsuperscript{18} The $B_{\text{max}}$ of $[^3]$HWAY100635 specific binding sites was 50-60% higher than that of $[^3]$H8-OH-DPAT in the same membrane preparations from various regions (hippocampus, septum, cerebral cortex).\textsuperscript{18a} Furthermore, the relationship between the $[^3]$HWAY100635 binding (total receptor density) and of $[^3]$H8-OH-DPAT binding (high affinity 5-HT\textsubscript{1A} binding sites only) in rat brain seems to depend upon the brain region.\textsuperscript{18b} In other words: if adrenalectomy (or another disease state) causes a shift from a low affinity state to a high affinity state of 5-HT\textsubscript{1A} receptors, this can be detected with a radiolabelled 5-HT\textsubscript{1A} receptor agonist, but not with $[^3]$HWAY100635 or $[^{11}]$CWAY100635.

Unfortunately, the partial 5-HT\textsubscript{1A} receptor agonist, $[^{11}]$CORG13502, was found to be unsuitable for in vivo imaging of (central) 5-HT\textsubscript{1A} receptors. Probably, the intrinsic activity, or unfavourable in vivo kinetic properties, of this compound undermine its
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ability to exert specific binding. In the present study, changed 5-HT<sub>1A</sub> receptor densities due to adrenalectomy did not result in altered brain uptake of the radioligand [<sup>11</sup>C]WAY100635. This may be due to the inability of [<sup>11</sup>C]WAY100635 to discriminate between the high affinity and low affinity state of 5-HT<sub>1A</sub> receptors. A radiolabelled 5-HT<sub>1A</sub> receptor agonist may differentiate between these two affinity states.

7.5 Experimental Section

General. For general remarks see Section 2.4. Log D values were calculated with Pallas version 1.2.

Materials. [<sup>11</sup>C]CH<sub>3</sub>I was produced from [<sup>11</sup>C]CO<sub>2</sub> (14N (p,α) <sup>11</sup>C nuclear reaction with 17 MeV protons) using an Anatech robotic system, yielding 15 GBq [<sup>11</sup>C]CH<sub>3</sub>I with a specific activity of more than 1000 Ci/mmol. The O-desmethyl precursors of ORG13502 and WAY100635 were prepared as described in Chapter 6 (section 6.5). 8-OH-DPAT (8-hydroxy-2-(N,N-di-n-propylamino)tetrinal) was synthesized in our laboratory according to published procedures.\(^9\)

Preparation of [<sup>11</sup>C]ORG13502. A solution of 1 mg (2.6 µmol; free base) desmethyl precursor and 0.3 mg (2.7 µmol) t-BuOK in 0.5 mL acetonitrile was prepared at least 30 min before adding [<sup>11</sup>C]CH<sub>3</sub>I. The methyliodide was trapped in the reaction vessel at 0 °C after which the reaction mixture was heated at 110 °C for 5 min in an oil bath. After 1 min of cooling an aliquot of 50 µL of the solution was evaporated to dryness under reduced pressure at 50 °C. The residue was dissolved in 1.0 mL HPLC-eluent, which consisted of methanol/10 mM phosphate buffer pH 7.4 65/35 (v/v). The reaction mixture was applied on a C-18 Reversed Phase column (Chrompack; 150 × 4.6 mm, 5 µm). Using a flow rate of 2 mL/min, [<sup>11</sup>C]ORG13502 was eluted after 8 min. After evaporation of the eluent under reduced pressure at 50 °C, [<sup>11</sup>C]ORG13502 was dissolved in saline to prepare it for injection. The radioligand was obtained in a radiochemical yield of ~60% (from [<sup>11</sup>C]CH<sub>3</sub>I, corrected for decay) with a radiochemical purity > 99%. The total synthesis time was 45 min and the specific activity was > 300 Ci/mmol.

Preparation of [<sup>11</sup>C]WAY100635. A similar procedure as for the synthesis of [<sup>11</sup>C]ORG13502 was employed. After heating the reaction mixture in an oil bath, an aliquot of 50 µL was dissolved in 1.0 mL HPLC-eluent (ethanol/water 55/45 (v/v)). The reaction mixture was applied on a semi-preparative C-8 Reversed Phase Column (Waters µBondapak; 300 × 7.8 mm, 5 µm) and [<sup>11</sup>C]WAY100635 was collected after 8 min, using a flow rate of 5 mL/min. [<sup>11</sup>C]WAY100635 was diluted with saline in order to prepare it for injection. The desired compound was obtained in a radiochemical yield of
~60% (from $^{11}$CMeI, corrected for decay) with a radiochemical purity > 99%. The specific activity of $^{11}$CWAY100635 was > 1000 Ci/mmol at the end of the 30-min radiosynthesis.

**Tissue distribution studies.** Protocols of the animal experiments were approved by a local ethics committee as is prescribed by the Law on Animal Experiments of The Netherlands. Male Wistar rats weighing 200-250 g were used. If required, rats were adrenalectomized (ADX) 1 or 6 days before the experiments. Before administration of the radioligand, the rats were treated either with saline (control group n = 8) or with 0.5 mg/kg 8-OH-DPAT (blocking experiments, n = 4) by intravenous (iv) injection in the tail vein. After ca. 2 min, 100 µCi of the radioligand was injected in a volume of 0.3 mL saline. Rats were killed by decapitation 60 min after injection. The brain was rapidly removed and the uptake of $^{11}$C was measured in striatum, frontal cortex, occipital cortex, hippocampus, thalamus, medulla oblongata, cerebellum, raphe nuclei and septum. The amount of radioactivity was expressed as a differential absorption ratio [DAR = (counts per min recovered/g tissue)/(counts per min injected/g body weight)]. The concentration of the radioligand that was specifically bound was calculated as the [radioactivity content (brain tissue)]/[radioactivity content (cerebellum)].

**Metabolism of $^{11}$CORG13502.** A heart-catheterized rat was injected with 100 µCi $^{11}$CORG13502 under anaesthesia. Blood-samples (200 µL) were taken at different time intervals, diluted with acetonitrile (1/1 v/v) and centrifuged (10,000 g, 2 min). The supernatant was injected onto a HPLC-system using a Waters RCM C-18 column (100 × 8 mm, 5 µm) which was eluted with acetonitrile/ 65 mM acetate buffer pH 6.5 55/45 (v/v), flow rate 2 mL/min. HPLC-samples were collected every 30 s and the radioactivity content was determined with a LKB Compu Gamma counter (Table 7.2)

**Statistics.** Differences between the 8-OH-DPAT treated group and the vehicle treated group were analyzed with the Student’s t-test (Table 7.1). Differences between the control groups and 8-OH-DPAT pretreated groups, in normal and ADX animals were analyzed using One Way Analysis of Variance (ANOVA) followed by Bonferroni’s t-test (Table 7.3).

**Acknowledgments.** Dr. Philip Elsinga and Ton Visser (PET-centre, University Hospital, Groningen, The Netherlands) are gratefully acknowledged for their assistance in operating the robotic system. We thank Dr. Durk Dijkstra for synthesizing 8-OH-DPAT.
7.6 References