Synthesis and Preclinical Evaluation of Novel PET Probes for P-Glycoprotein Function and Expression

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P-glycoprotein (P-gp) is an ATP-dependent efflux pump protecting the body against xenobiotics. A P-gp substrate (7) and an inhibitor (6) were labeled with $^{11}$C, resulting in potential tracers of P-gp function and expression. Methods: 6 and 7 were labeled using $^{11}$C. $^{11}$C-verapamil was prepared as published previously, using $^{11}$C-methyl triflate. MicroPET scans (with arterial sampling) and biodistribution studies were performed in rats pretreated with saline, cyclosporin A (CsA), 50 mg/kg, or cold 6 (15 mg/kg).

Results: The radiochemical yields of $^{11}$C-6 and $^{11}$C-7 were approximately 30% with a total synthesis time of 45 min. Cerebral distribution volumes (DV) of $^{11}$C-6 (2.35 ± 0.11) and $^{11}$C-7 (1.86 ± 0.15) in saline-treated rats were higher than of $^{11}$C-verapamil (0.64 ± 0.12). DVs of $^{11}$C-7 and $^{11}$C-verapamil were significantly increased by CsA (to 5.26 ± 0.14 and 5.85 ± 0.32, respectively). The DV of $^{11}$C-6 was reduced by cold 6 (to 1.65 ± 0.03). Its uptake was also reduced (up to 67%) in several peripheral organs that express P-gp. Conclusions: $^{11}$C-7 is a novel tracer of P-gp function with higher baseline uptake than $^{11}$C-verapamil. Upregulation of P-gp function in response to treatment (which is hard to detect with $^{11}$C-verapamil) may be detectable using $^{11}$C-7 and PET. Because $^{11}$C-6 shows specific binding in target organs, this compound is the first PET tracer allowing measurement of P-gp expression.

Introduction

P-glycoprotein (P-gp) is an ATP-dependent efflux pump with a very high expression in several organs, particularly the blood—brain barrier (BBB) and the gastro-intestinal tract. This transport protein protects the mammalian body against xenobiotics. Several therapeutic drugs are substrates for P-gp, resulting in a low residual uptake of such compounds in the human brain, even though the compounds in question may have the optimal lipophilicity for passage of the BBB.1,2 Reduced P-gp function in the BBB has been observed in neurodegenerative diseases,3-5 and this function may also be altered by stress.6,7 P-gp expression is one of the mechanisms underlying resistance of patients to antiepileptic, anti-HIV, and antidepressant drugs.8,9 Moreover, P-gp expression is a possible mechanism causing drug resistance of human tumors.10-12 Because many drugs are substrates for P-gp and because the efflux pump is saturable, the combined prescription of different P-gp substrates may cause unexpected side effects because of increased entry into the brain, altered absorption in the gastrointestinal tract, or impaired renal excretion of compounds from the cocktail.13-18 For these reasons, there is a considerable interest in the noninvasive quantification of P-gp expression and function using PET.

In a recent publication, several novel P-gp modulating molecules were described.19 These compounds are potent and structurally different from existing P-gp modulators. 6,7-Dimethoxy-2-[3-(4-methoxy-3,4-dihydro-2H naphthalen- (1E)-ylidene)propyl]-1,2,3,4-tetrahydro-isoquinoline (later named 11C-6, compound 6 in Figure 1) is a P-gp inhibitor, whereas 6,7-dimethoxy-2-[3-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-1-y1)propyl]-1,2,3,4-tetrahydro-isoquinoline (later named MC-266, compound 7 in Figure 1) is a P-gp substrate. Because both molecules contain methoxy groups (Figure 1), they can be labeled with $^{11}$C, which will result in novel potential PET tracers of P-gp expression and function.

Several radiolabeled substrates for quantification of P-gp function are already available. These include $^{11}$C-verapamil,20-21 $^{11}$C-carvedilol,22 $^{11}$C-GR218231,23 and $^{11}$C-N-des-methyl-loperamide.24 However, the detection of subtle changes in function (particularly an upregulation of P-gp) is problematic because of the very large capacity of the pump, resulting in a very low brain uptake of radiolabeled substrates under most conditions. Thus, there is opportunity for the development of novel positron-emitting ligands for P-gp with improved kinetics. Visualization of P-gp expression using a radiolabeled inhibitor has not yet been reported, although the synthesis of $^{11}$C-laniquidar, a putative P-gp inhibitor, has been described.25 Independent assessment of pump function and expression (using a radiolabeled substrate and inhibitor) may provide novel insights in the pathophysiology of disease.
Moreover, radiolabeled inhibitors may allow assessment of the occupancy of inhibitor sites on the P-gp molecule by novel (nonradioactive) drugs. Here, we present the radiochemical synthesis of $^{11}$C-6 and $^{11}$C-7 besides a preliminary evaluation of these compounds in rodents, using microPET.

**Results**

**MicroPET Images.** MicroPET images of animals acquired after injection of $^{11}$C-verapamil, $^{11}$C-7, and $^{11}$C-6 are presented in Figure 2. Uptake of $^{11}$C-verapamil in the CNS of saline-treated control animals was very low. In baseline scans, the brain appeared as a “cold spot”, surrounded by areas with superior levels of radioactivity, such as muscle tissue and salivary glands. The sickle-shaped organs in front of the brain, which demonstrated very high $^{11}$C-verapamil uptake, are the Harderian glands. After pretreatment of animals with CsA (50 mg/kg), the cerebral SUV of the tracer was dramatically increased and the entire brain was visualized (Figures 2a and 3).

Very similar results were obtained with the P-gp modulator $^{11}$C-7. However, in this case, a faint outline of the brain was already visible in baseline scans because the SUV of $^{11}$C-7 in the CNS of saline-treated control animals was higher than that of $^{11}$C-verapamil (compare Figures 2b and 3). After pretreatment of animals with CsA (50 mg/kg), the cerebral SUV of $^{11}$C-7 was strongly increased and the $^{11}$C-7 scans acquired the same appearance as $^{11}$C-verapamil scans (Figure 2b).

The baseline SUV of $^{11}$C-6 into rat brain was higher than that of $^{11}$C-verapamil and $^{11}$C-7 (Figure 3). Thus, the brain was fairly well-visualized in a $^{11}$C-6 scan at baseline conditions (Figure 2c). After pretreatment of animals with nonradioactive 6 (15 mg/kg), tracer uptake was reduced and the brain-to-background contrast was impaired (Figure 2c and Figure 3).

**Kinetics of Radioactivity within Brain.** Cerebral kinetics of radioactivity after injection of $^{11}$C-verapamil, $^{11}$C-7, and $^{11}$C-6 is presented in Figure 3. In saline-treated control animals, hardly any $^{11}$C-verapamil entered the brain. Maximal uptake was already observed in the first frame (i.e., 15 s after tracer injection) and was followed by an exponential washout. After pretreatment of animals with cyclosporin A (50 mg/kg), the cerebral uptake of $^{11}$C-verapamil was strikingly increased. Maximal uptake now occurred after 8–12 min (Figure 3a). The effect of CsA treatment (brain uptake in treated animals divided by brain uptake in control rats) was maximal after 20–40 min. At such intervals, CsA caused a 9-fold increase of the entry of $^{11}$C-verapamil into the brain (SUV increased from 0.15 ± 0.03 to 1.34 ± 0.07 at 25 min). The area-under-the-curve (AUC) of $^{11}$C-verapamil in the brain (0–60 min) was increased 7.2-fold by CsA.

Uptake of $^{11}$C-7 in the brain of control animals was higher than uptake of $^{11}$C-verapamil (Figure 3b). Both in control animals and in animals treated with CsA, maximal uptake

![Figure 1. Chemical structures of the radiolabeled compounds. The position of the $^{11}$C-label is indicated by the square.](image-url)
was observed in the second frame (i.e., 30 s after tracer injection) and was followed by an exponential washout. After pretreatment of animals with CsA (50 mg/kg), the cerebral uptake of $^{11}$C-$^{7}$ was strongly increased (Figure 3b). The effect of CsA treatment on the brain uptake of $^{11}$C-$^{7}$ (data points from upper curve in Figure 3b divided by data points of the lower curve) was maximal after 20–40 min. At these time points, CsA caused a 2.5-fold increased entry of $^{11}$C-$^{7}$ into the rat brain (SUV increased from 0.88 (0.07 to 2.17 (0.10 at 25 min). AUC of $^{11}$C-$^{7}$ uptake in the brain showed a 2.2-fold increase.

Both in control animals and in rats treated with nonradioactive $^{6}$, maximal brain uptake of $^{11}$C-$^{6}$ was already reached at 30 s after tracer injection. After pretreatment of animals with "cold" $^{6}$ (15 mg/kg), the cerebral uptake of $^{11}$C-$^{6}$ was decreased, particularly at intervals greater than 30 min (Figure 3c). The effect of pretreatment with cold compound on $^{11}$C-$^{6}$ uptake was maximal in the last 2 frames, i.e., from 40 to 60 min post injection. At these intervals, pretreatment resulted in a statistically significant, 23% decline of tracer SUV (from 0.88 ± 0.04 to 0.68 ± 0.03 at 56 min, $p < 0.01$). AUC of $^{11}$C-$^{6}$ in the brain was 17% decreased after pretreatment with cold compound, but this decrease did not reach statistical significance.

**Kinetics of Radioactivity in Plasma.** Plasma kinetics of radioactivity after injection of $^{11}$C-verapamil, $^{11}$C-$^{7}$, and $^{11}$C-$^{6}$ is presented in Figure 4. During the study interval of 60 min, $^{11}$C-verapamil was cleared from the circulation to a greater extent than either $^{11}$C-$^{7}$ or $^{11}$C-$^{6}$. Plasma SUVs at 60 min post injection were 0.18 ± 0.01, 0.34 ± 0.03, and 0.49 ± 0.05 for $^{11}$C-verapamil, $^{11}$C-$^{7}$, and $^{11}$C-$^{6}$, respectively.

Drug treatment did not affect the clearance of $^{11}$C-verapamil, $^{11}$C-$^{7}$, or $^{11}$C-$^{6}$ from rat plasma. The area-under-the-curve (AUC) for $^{11}$C-verapamil was 100 ± 8% in control animals and 110 ± 6% in animals treated with CsA. For $^{11}$C-$^{7}$, it was 100 ± 7% in control animals and 97 ± 3% in animals treated with CsA, and for $^{11}$C-$^{6}$, 100 ± 6% in control animals and 102 ± 9% in animals treated with cold compound (mean ± s.e.m.). None of these differences was statistically significant.

**Analysis of Tracer Kinetics.** Influx rate constants ($K_{i}$ values) and distribution volumes of the tracers in rat brain were calculated using Logan plots and data from arterial blood samples as input function. Cyclosporin A treatment resulted in highly significant increases of the cerebral distribution volume (9-fold, from 0.64 ± 0.12 to 5.85 ± 0.32, $p < 0.0001$) and of $K_{i}$ (2.8-fold, from 0.22 ± 0.04 to 0.81 ± 0.12, $p < 0.002$) of $^{11}$C-verapamil, as described previously.

CsA treatment increased the cerebral distribution volume of $^{11}$C-$^{7}$ (3-fold, from 1.86 ± 0.15 to 5.26 ± 0.14, $p < 0.0001$). The influx rate constant ($K_{i}$) of $^{11}$C-$^{7}$ into brain tissue was increased as well (2.5-fold, from 0.18 ± 0.03 to 0.64 ± 0.08, $p < 0.002$). The cerebral distribution volume of $^{11}$C-$^{7}$ in
saline-treated control animals (1.86 ± 0.15) was significantly higher than that of \(^{11}\text{C}\)-verapamil (0.64 ± 0.12, \(p = 0.0002\)).

Pretreatment of animals with nonradioactive \(6\) (15 mg/kg) resulted in a significant (30%) decrease of the cerebral distribution volume of \(^{11}\text{C}\)-\text{C}6 (from 2.35 ± 0.11 to 1.65 ± 0.03, \(p = 0.005\)). A decrease of \(K_1\) was also noted (from 0.90 ± 0.08 to 0.45 ± 0.45), but this trend failed to reach statistical significance because of a large standard error in the treated group. The cerebral distribution volume of \(^{11}\text{C}\)-\text{C}6 in saline-treated control animals (2.35 ± 0.11) was much higher than that of \(^{11}\text{C}\)-\text{C}7 (0.64 ± 0.12, \(p < 0.0001\)) and slightly higher than that of \(^{11}\text{C}\)-\text{C}7 (1.86 ± 0.15, \(p < 0.05\)).

**Biodistribution Data.** Pretreatment of animals with cyclosporin A (CsA) caused a highly significant increase of \(^{11}\text{C}\)-verapamil uptake (SUV) in rat brain (11- to 22-fold depending on the region, Table 1). CsA also increased the uptake or retention of \(^{11}\text{C}\)-verapamil-derived radioactivity in several peripheral organs, viz. kidney, liver, lung, pancreas, and spleen (Table 1).

Pretreatment with CsA increased the uptake of \(^{11}\text{C}\)-\text{C}7 into rat brain 2.0- to 3.5-fold, depending on the region (Table 1). However, CsA did not affect the concentration of \(^{11}\text{C}\)-\text{C}7-derived radioactivity in any peripheral organ (Table 1). The baseline uptake of \(^{11}\text{C}\)-\text{C}7 into rat brain was significantly higher than the baseline uptake of \(^{11}\text{C}\)-verapamil. SUV values between 0.3 and 0.4 were observed in most regions, whereas for \(^{11}\text{C}\)-verapamil, they were between 0.03 and 0.04 (Table 1).

After pretreatment of animals with nonradioactive \(6\), the uptake of \(^{11}\text{C}\)-\text{C}6 in many brain areas was reduced (by 30–40%, Table 1). Such pretreatment caused also a striking decline of tracer uptake in colon, duodenum, ileum, and spleen (by 60%, 67%, 53%, and 35%, respectively). A trend toward decreased uptake of \(^{11}\text{C}\)-\text{C}6 was noticed in pancreas and kidney, but particularly in the pancreas, this failed to reach statistical significance because of a large variability in the control group. Pretreatment with cold \(6\) increased tracer SUV in some peripheral nontarget organs, such as heart, lungs and red blood cells (Table 1).

**Discussion**

The molecules which were labeled with \(^{11}\text{C}\) in the present study (6, 7) were previously screened for their ability to interact with human Pgp, using the following in vitro systems:19 (i) monolayer of Caco-2 cells grown on permeable filters, and (ii) MCF7/adr cells.
At a concentration of 20 μM, 6 increased the intracellular accumulation of doxorubicin in the MCF7/adr cell line 5.7-fold and potentiated the antiproliferative effect of 5 μM doxorubicin from 5 ± 1% to 95 ± 2%. The ratio of drug transport through Caco-2 monolayers in the basolateral–apical and apical–basolateral directions (P_{app} BA/AB) was 1.6, i.e., less than 2, and the compound did not activate ATPase activity within the monolayer. On the basis of these data, 6 was classified as a P-gp inhibitor.19

Compound 7 (at a concentration of 20 μM) increased the intracellular accumulation of doxorubicin in MCF7/adr cells 4.8-fold and potentiated the antiproliferative effect of 5 μM doxorubicin from 5 ± 1% to 85 ± 2%. P_{app} BA/AB for this compound was 18, i.e., much greater than 2, and in contrast to 6, 7 strongly activated ATPase activity within Caco-2 cells. Thus, 7 was classified as a P-gp substrate.19

**Brain Uptake and Kinetics of the Tracers.** Both the micro-PET images (Figure 2) and the cerebral time–activity curves (Figure 3) indicated that the P-gp substrate 11C-7 behaved similarly to 11C-verapamil. Low uptake was observed in saline-treated control animals, and uptake of radioactivity in the brain was strongly increased after pretreatment of animals with the P-gp modulator CsA. Our time–activity curves for 11C-verapamil in rat brain after pretreatment of animals with CsA were qualitatively similar to those reported for (R)-11C-verapamil in animals pretreated with tariquidar using a Focus 220 microPET camera31 and to those for 11C-verapamil reported previously using an ECAT Exact HR.30

However, the images, curves, and biodistribution data (Table 1) also indicated that the uptake of 11C-7 in the brain of control animals was higher than that of 11C-verapamil. This higher brain uptake could be due to: (i) better delivery to the brain after intravenous injection, (ii) a greater influx rate constant, and (iii) a lower P-gp mediated drug efflux of 11C-7 as compared to 11C-verapamil. Because 11C-7 and 11C-verapamil showed similar influx rate constants into rat brain (0.18 ± 0.03 and 0.22 ± 0.04, respectively), the second explanation is not correct. The first mechanism appears to play a role because 11C-7 reaches higher concentrations in rat brain than 11C-verapamil. The data from the arterial blood samples (Figure 4) indicate an area-under-the-curve for 11C-7, which is 2.3-fold higher than that of 11C-verapamil. Because the baseline uptake of 11C-7 into rat brain is not 2.3-fold, but rather 10-fold higher than the baseline uptake of 11C-verapamil (Table 1), there should be an additional mechanism involved, viz. a difference in pumping efficiency. CsA increased the brain uptake of 11C-7 to a smaller extent than the brain uptake of 11C-verapamil (2.5-fold and 9-fold, respectively).

Although the brain uptake of 11C-7 is less sensitive to modulation of P-gp with CsA than the brain uptake of 11C-verapamil, the elevated baseline uptake of 11C-7 could be advantageous under certain circumstances. An up-regulation of P-gp function (e.g., in epilepsy) is hard to detect with 11C-verapamil because the uptake of this radiopharmaceutical in the normal brain is already very low. Increases of P-gp function (and the accompanying decreases of the DV of a radiolabeled P-gp substrate) may be better detectable with 11C-7, although further studies (e.g., in animal models of epilepsy) are required to test this.

The P-gp inhibitor 11C-6 showed an appreciable uptake in control brains that was significantly (23%) reduced after pretreatment of rats with cold compound (Figures 2 and 3).

These results, and the biodistribution data discussed below, suggest that specific binding of 11C-6 to the P-gp molecule can be detected with PET, although the target-to-nontarget ratio (particularly in the brain) is small.

**Plasma Kinetics.** Data from the arterial blood samples (Figure 4) indicated that 11C-6 and 11C-7 were cleared to a smaller extent from plasma than 11C-verapamil. Particularly at intervals greater than 10 min, radioactivity levels in the circulation were higher after injection of the novel compounds than after injection of 11C-verapamil. This difference is also apparent from the biodistribution data, which were acquired 80 min after tracer injection (Table 1).

The plasma time–activity curves and calculated AUC values showed that pretreatment of animals with CsA or nonradioactive 6 did not significantly affect clearance of the injected radioactivity from the circulation (Figure 4). Changes of brain SUV that we observed (Figure 3) are therefore not related to altered tracer delivery but to interactions of the test drugs with the P-gp pump. To substantiate this claim, we have performed a kinetic analysis of tracer uptake into the rodent brain.

**Kinetic Analysis of Uptake Data.** A graphical analysis of our uptake data using the Logan method30 indicated that the distribution volume of 11C-7 in control brains was higher than that of 11C-verapamil (1.86 ± 0.15 and 0.64 ± 0.12, respectively). Because the K1 values for 11C-7 and 11C-verapamil were not significantly different (0.18 ± 0.03 and 0.22 ± 0.04, respectively), the graphical method suggests that 11C-7 is pumped less efficiently out of the rat brain than 11C-verapamil.

The distribution volumes of both 11C-7 and 11C-verapamil were strongly increased after pretreatment of animals with CsA (5.26 ± 0.14 and 5.85 ± 0.32), as should be expected for P-gp substrates. These increases in DV could be attributed mainly to increased influx rate constants of 11C-7 and 11C-verapamil into the brain, as was reported previously for (R)-11C-verapamil in animals pretreated with tariquidar.31

The distribution volume of 11C-6 in rat brain was decreased after pretreatment of animals with cold compound (from 2.35 ± 0.11 to 1.65 ± 0.03), suggesting that the decreased uptake which was noted in the time–activity curves (Figure 3) reflects saturation of an inhibitor binding site on the P-gp protein.

**Biodistribution Data.** Our biodistribution data for 11C-7 are similar to those reported previously for 11C-verapamil.32 Brain uptake (SUV) of the compound was significantly increased after pretreatment of animals with CsA, whereas tissue uptake in peripheral organs was not significantly affected (Table 1). In contrast to data reported previously for 11C-verapamil,32 CsA now increased the retention of 11C-verapamil not only in the brain but also in some peripheral organs such as kidney, liver, lung, pancreas, and spleen (Table 1). The difference between the present and the previously reported data may be due to strain differences. In the previous study, nude animals were used (HSD Ham RNU rnu), whereas the current study employed normal Wistar rats. In the Wistar strain, CsA appears to affect retention of the tracer in excretory organs.

In rats, there are two mdr1 genes involved in the transport of xenobiotics: mdr1a and mdr1b. The highest concentrations of mdr1a mRNA are found in the gastrointestinal tract. Expression levels of mdr1a mRNA in other target tissues are less than 10% of those in the ileum. The highest expression of mdr1b mRNA also occurs in the gastrointestinal tract, but
the expression of that gene in other target tissues is only 3-fold lower than in the gut.33 P-gp is located at the apical plasma membrane in the intestinal epithelium. To enter the blood, dietary xenobiotics must pass both the apical and basolateral membranes of epithelial cells. Although lipophilic substances may readily diffuse across the apical plasma membrane, P-gp drives such compounds back into the intestinal lumen, preventing their absorption into blood.34 According to several studies that were performed in healthy rats, there is a close relationship between mdr gene expression at the mRNA and protein levels.35–37 Thus, the data reported by Brady et al.33 suggest that the tissue concentration of P-glycoprotein is highest in the rat intestine, whereas lower amounts of protein will be present in other target tissues such as the brain. In the current study, we observed a tissue distribution of the specific binding of 11C-6 that corresponds to the known expression levels of P-gp. The highest amounts of specific binding (53–67% of total radioactivity uptake) were observed in colon, duodenum, and ileum (Table 1). A lower fraction of specific binding (35% of total radioactivity uptake) occurred in the spleen, and the lowest fraction (17% to 34% of total tissue uptake) was observed in areas of the brain such as pons, parietal, temporal, and occipital cortex (Table 1). Pretreatment of animals with cold compound tended to reduce the uptake of 11C-6 in the pancreas as well (by 33%), but this tendency was not statistically significant because of a large variability of pancreatic uptake in the control group. Little information is available on mdr expression in rat spleen and pancreas, but P-gp-mediated transport of the substrate 11C-GRAV218231 has been observed in these organs.23 Thus, our biodistribution data suggest that it is possible to visualize the local expression of the P-gp protein with a radiolabeled inhibitor such as 11C-6 and PET. However, the low specific binding fraction of 11C-6 in the brain (one-fourth to one-third of the total tissue uptake of radioactivity) also indicates that inhibitors with higher affinity for P-gp may be required for quantitative imaging of P-gp expression at the blood-brain barrier.

**Conclusion**

11C-7 is a novel tracer of P-gp function with higher baseline uptake than 11C-verapamil. Upregulation of P-gp function in response to treatment may be detectable using 11C-7 and PET. 11C-6 is a unique tracer of P-gp expression, showing specific binding in the brain and several peripheral target organs. However, a derivative with higher affinity for the pump may be required for quantitative imaging. The prospect of independent assessment of pump function and expression is quite exciting because this may allow study of the relationship between P-gp function and expression in psychiatric disorders and neurodegenerative diseases.

**Experimental Section**

Column chromatography was performed with 1:30 Merck silica gel 60 Å (63–200 μm) as the stationary phase. Melting
points were determined in open capillaries on a Gallenkamp electrophoretic apparatus. 1H NMR spectra were recorded in CDCl3 at 300 MHz on a Varian Mercury-VX spectrometer. All spectra were recorded on the free bases. All chemical shift values are reported in ppm (δ). Recording of mass spectra was done on an HP6890–5973 MSD gas chromatograph/mass spectrometer; only significant m/z peaks, with their percentage of relative intensity in parentheses, are reported. All spectra were in accordance with the assigned structures. ESI-MS analyses were performed on an Agilent 1100 LC/MSD trap system VL. Compound 3 has not been characterized because of its instability. Purity of compound 2 was established by combustion analysis conforming a purity ≥95%. Purity of 4 and 5 was established by combustion analysis of the corresponding hydrochloride salts, confirming a purity ≥95%.

Chemistry, Test Drugs, and Radiopharmaceuticals. Cyclopentorin A (Sandozmin, 50 mg/mL solution in polyoxyethylated Ricinus oil) was a product of Sandoz.

Compounds 6 and 7 were synthesized as described previously (see Figure 1 for chemical structures). The synthesis of desmethyl-6 and desmethyl-7 is depicted in Scheme 1. Compound 4 was prepared by alkylation 6,7-dimethoxytetrahydroisoquinoline with compound 3. The key intermediate 3 was synthesized by Grignard’s reaction between cyclopropylMgBr and tetralone derivative 2. Compound 3 was obtained by kinetic control of the reaction; because of its instability, it has not been characterized. Compound 2 was prepared by employing CH3SO2Cl, for masking the hydroxy substituent of starting compound 1. Compound 5 was prepared by catalytic reduction (Pd/C 5%) of compound 4 under hydrogen pressure. 11C]-Verapamil was produced from desmethyl-verapamil as previously described. The radiochemical yield was >60% (EOB, based on 11C-methyl triflate), radiochemical purity >99%, and the specific activity > 5 TBq/mmol. Injected doses (30 to 60 MBq) are indicated in Table 1. 11C]-6 and 11C]-7 were prepared from 11C-methyl iodide and desmethyl-6 (compound 4 in Scheme 1) or desmethyl-7 (compound 5 in Scheme 1), respectively. The radiochemical yield of both compounds was 30%, radiochemical purity >98%, and the specific activity >100 TBq/mmol. The injected dose of 11C]-6 varied between 8 and 15 MBq, as indicated in Table 1. The injected dose of 11C]-7 was 25 to 40 MBq.

Animals. The animal experiments were performed by licensed investigators in compliance with the Law on Animal Experiments of The Netherlands. The protocol was approved by the Committee on Animal Ethics of the University of Groningen. Male Wistar rats were maintained at a 12 h light/12 h dark regime and were fed standard laboratory chow ad libitum. Body weights of the animals at the time of microPET scanning are indicated in Table 1. Before any animal was scanned, a cannula was placed into one of its femoral arteries under isoflurane anesthesia. This cannula was later used for blood sampling. Pretreatment of the animals (either with saline or with one of the test drugs) was done by injection of the appropriate drug solution through a lateral tail vein. Cold test drugs were done by injection of the appropriate drug solution through the penile vein after careful positioning of the animals inside the scanner. Tracer injection was performed through the penile vein after careful positioning of the animals into the microPET scanner.

MicroPET scanning. Two rats were scanned simultaneously in each scan session, using a Siemens/Concorde microPET camera (Focus 220). A list mode protocol was used (76 min, brain in the field-of-view). Data acquisition was started during tracer injection of the first rat; the second animal was injected 16 min later.

After injection of radioactivity into each rat, 14 arterial blood samples (volume 0.1–0.15 mL) were drawn, using a standard protocol (at 15, 30, 45, 60, 75, and 90 s, 2, 3, 5, 7.5, 10, 15, 30, and 60 min after injection, respectively). Plasma was obtained from these samples by short centrifugation (5 min in Eppendorf-type centrifuge at maximum speed). Radioactivity in plasma samples (25 μL) was determined using a calibrated γ counter.

List mode data were reprocessed into a dynamic sequence of 4 × 60 s, 3 × 120 s, 4 × 300 s, 3 × 600 s frames after tracer injection. The data were reconstructed per time frame employing an interactive reconstruction algorithm (OSEM2D with Fourier rebinning, 4 iterations, 16 subsets). The final data sets consisted of 95 slices with a slice thickness of 0.8 mm and an in-plane image matrix of 128 × 128 pixels. Voxel size was 0.5 mm × 0.5 mm × 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. A separate transmission scan was acquired for attenuation correction. This scan was performed right before the emission scan.

Biodistribution Studies. After the scanning period, the anesthetized animals were terminated. Blood was collected, and plasma and a cell fraction were obtained from the blood sample (5 mL) by short centrifugation (5 min at 1000 g). Several tissues (see Table 1) were excised. All tissue samples were weighed. Radioactivity in tissue samples was measured using a γ counter, applying a decay correction. The results were expressed as dimensionless standardized uptake values (SUVs). The parameter SUV is defined as: [tissue activity concentration (MBq/g) × body weight (g)/injected dose (MBq)].

MicroPET Data Analysis. Three-dimensional regions of interest (3D-ROIs) were manually drawn around the entire brain. Time-activity curves (TACs) and volumes (cm3) of the ROIs were calculated using standard software (AsiPro VM 6.2.5.0, Siemens-Concorde, Knoxville, TN). SUVs were calculated by the formula shown in the previous paragraph, assuming a specific gravity of 1 g/mL for brain tissue and blood plasma.

Dynamic PET data were analyzed using plasma radioactivity from arterial blood samples as input function and a graphical method according to Logan. Software routines for MatLab 7 (The MathWorks, Natick, MA), written by Dr. ATM Willemens (University Medical Center Groningen), were employed for curve fitting. The Logan fit was started at 20 min and the parameter for cerebral blood volume was fixed at 0.036. The cerebral distribution volume (DV) and the influx rate constant (Ki) of the tracer were estimated from the curve fit.

Statistical tests. Differences between groups were analyzed using Student’s t-test. A dual-tail probability smaller than 0.05 was considered statistically significant.

5-[3-(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)propyl]-6,7,8-tetrahydro-naphthalen-1-ol (4). A mixture containing crude intermediate 3 (1 mmol), 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (3 mmol), and anhydrous Na2CO3 (0.50 mmol) in 20 mL DMF was refluxed for 4 h. The solvent was evaporated, and the residue was partitioned between H2O (20 mL) and CHCl3 (30 mL). The organic layer was dried (Na2SO4) and concentrated in vacuo. The crude residue was chromatographed on a silica gel column with CHC13/AcOEt 7:3 obtaining compound 4 as colorless oil (43% yield). GC-MS: m/z 380 (M+ + 1, 1), 379 (M+ 3), 206 (100%). 1H NMR δ 1.82–1.90 (m, 2H, CH2CH(CH2CH2CH), 2.47–2.95 (mm, 12H, CH2CH(CH2CH2CH), NCH2CH2tetrahydroisoquinoline CHCH2(NHCH2), 3.66 (s, 2H, NCH2tetrahydroisoquinoline), 3.83 and 3.84 (2s, 6H, 2 of CH3), 7.17 (m, 5H, aromatic), 9.50–9.80 (br s, 1H, OH, D2O exchange).

5-[3-(6,7-Dimethoxy-3,4-dihydro-1H-isoquinolin-2-yl)propyl]-6,7,8-tetrahydro-naphthalen-1-ol (5). Desmethyly-6 (10 mmol) were solubilized in EtOH (100 mL) and hydrogenated in the presence of 5% palladium on activated carbon (200 mg) at normal pressure and room temperature until theoretical uptake was accomplished. The reaction mixture was filtered through celite and evaporated to dryness to obtain desmethyly-7. The crude product was purified on a silica gel column (petroleum ether/CH2Cl2 9:1), obtaining compound 5 as colorless oil (yield 90%).
HLB SepPak, followed by rinsing of the cartridge (twice) with MeCN were removed by passing the mixture through an Oasis solution (0.5 mg in 0.5 mL anhydrous DMSO) of desmethyl-


References


