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Influence of P-glycoprotein on brain uptake of [^{18}F]MPPF in rats

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Abstract

The aim of this study was to determine if the brain uptake of 4-(2'-methoxyphenyl)-1-[2'-(*N*-2"-pyridinyl)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine ([^{18}F]MPPF), a radioligand for the imaging of 5-HT_{1A} receptors, is influenced by the action of P-glycoprotein.

Anesthetized male Wistar rats were injected i.v. with the 5-HT_{1A} receptor antagonist [^{18}F]MPPF (2 MBq, S.A. > 110 TBq/mmol) after treatment with saline (controls) or with the 5-HT_{1A} receptor antagonist 1-(2'-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190) (2.5 mg/kg i.v.). After 60 min, the animals were sacrificed and 13 areas of the brain were dissected for ex vivo gamma counting. The regional distribution of radioactivity was also assessed in brain slices using a storage phosphor system. Modulation of P-glycoprotein was achieved by injection of cyclosporin A (50 mg/kg) 30 min prior to injection of [^{18}F]MPPF.

The distribution of ^{18}F -derived radioactivity corresponded to regional 5-HT_{1A} receptor density as known from autoradiography. Modulation of P-glycoprotein with cyclosporin A caused a 5- to 10-fold increase in the uptake of [^{18}F]MPPF. Tissue/cerebellum ratios in the brain correlated with receptor densities determined by in vitro autoradiography. Measurements of plasma radioactivity showed that the increased brain uptake of [^{18}F]MPPF is partially due to a rise in ligand delivery after treatment with cyclosporin A (area under the curve, AUC, increased by a factor of 1.8). Biodistribution experiments in wild type and *mdr1a*(-/-) knockout mice confirmed that [^{18}F]MPPF is a substrate for P-glycoprotein. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: 5-HT_{1A} receptor; Positron Emission Tomography (PET); P-glycoprotein

1. Introduction

Positron emission tomography (PET) and biodistribution studies in rats, cats, and monkeys have demonstrated that the regional distribution of 4-(2'-methoxyphenyl)-1-[2'-(*N*-2"-pyridinyl)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine ([^{18}F]MPPF) in the brain corresponds to 5-HT_{1A} receptor localization (Kung et al., 1996; Shiue et al., 1997; Le Bars et al., 1998; Ginovart et al., 2000). Recently, our group presented the first human data for [^{18}F]MPPF (Passchier et al., 1999, 2000a,b). Again, the radioactivity distribution in human CNS was in good agreement with known receptor localization and with previous PET studies, which employed [carbonyl- ^{11}C]WAY 100635 (Farde et al., 1998; Gunn et al., 1998; Ito et al., 1999). Shortly afterwards, these results were confirmed by researchers from Liège (Plenevaux et al., 1999), indicating that [^{18}F]MPPF is a

useful radioligand for clinical application and may be employed to measure the 5-HT_{1A} receptor occupancy of new drugs during phase 1 and 2 clinical trials.

Comparison of data obtained for [^{18}F]MPPF in rats with data for [carbonyl- ^{11}C]WAY 100635, showed that MPPF displays a six- to seven-fold lower uptake in the brain (data not shown). This difference must arise from the *p*-fluoro-benzyl group in MPPF since the compounds are otherwise identical (Scheme 1).

It is known that P-glycoprotein, an ATP-driven transmembrane efflux pump, which is present with high density in brain endothelium, has a high affinity for lipophilic molecules of moderate weight and size that contain cationic centers and planar aromatic domains (Abbott and Romero, 1996; Dellinger et al., 1992; Pawagi et al., 1994; Pearce et al., 1990; Schinkel et al., 1996; Van Asperen et al., 1996, 1997). Active transport back into the blood could result in a lower brain penetration of [^{18}F]MPPF than might be expected from its lipophilicity. The efflux action of P-glycoprotein can be inhibited by so-called modulators (e.g. cyclosporin A, verapamil, and PSC833) (Begley, 1992;

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Scheme 1. Characteristics of MPPF and WAY 100635.

Hendrikse et al., 1998; Hughes et al., 1998; Schinkel et al., 1995; Van Asperen et al., 1996). These compounds reduce P-glycoprotein functionality and thus prevent a radioligand from being expelled from the endothelial membrane back into the blood.

In rodents, two P-glycoproteins are present encoded by the *mdr1a* and *mdr1b* genes. Brain P-glycoprotein is expressed by the *mdr1a* gene (Borst and Schinkel, 1996; Schinkel et al., 1995, 1996; Van Asperen et al., 1996). The generation of mice with homozygously disrupted *mdr1a* genes [*mdr1a*(–/–) knockout mice] has made it possible to test if the brain uptake of a drug is affected by the action of P-glycoprotein (Schinkel et al., 1995, 1996; Van Asperen et al., 1996).

If uptake of the radiopharmaceutical in the brain is indeed limited by the action of P-glycoprotein, inhibition of this pump by a modulator could result in improved counting statistics within the CNS. A positive result will demonstrate the importance of taking the action of P-glycoprotein into account during the development of radioligands for CNS receptors and for 5-HT_{1A} receptor antagonists in particular.

The aim of the present study was to investigate if the introduction of the extra aromatic ring in MPPF results in a decreased brain uptake due to the efflux action of P-glycoprotein. To that purpose, biodistribution experiments were performed in rats with modulated P-glycoprotein functionality and in *mdr1a*(–/–) knockout mice.

2. Materials and methods

All solvents were of analytical grade and were obtained from Merck (Darmstadt, Germany) or Rathburn (Walkerburn, Scotland). Chemicals were purchased from Aldrich (Milwaukee, USA) and ACROS Chimica (Geel, Belgium). NAN-190 Hydrobromide and (*R*)-(+)-8-Hydroxy-DPAT were from Sigma (St. Louis, USA) Cyclosporin A (Sandimmune) was a product of Sandoz (Basle, Switzerland). Ketamine (Ketalar®) was purchased from Parke-Davis (Hoofddorp, The Netherlands) and xylazine (Rompun®) from Bayer (Leverkusen, Germany). 4-(2'-methoxyphenyl)-1-[2'-(*N*-2"-pyridinyl)-*p*-nitrobenzamido]-

ethylpiperazine (*p*-MPPNO₂), 4-(2'-methoxyphenyl)-1-[2'-(*N*-2"-pyridinyl)-*p*-fluorobenzamido]ethylpiperazine (*p*-MPPF), and {2-(4-(2-methoxyphenyl)-piperazin-1-yl)-ethyl}-piperidin-2-yl-amine (WAY 100634) were prepared according to a previously described method (Zhuang et al., 1994). C18 SepPak light and OASIS cartridges were obtained from Waters Chromatography Division (Milford, USA). Supelclean LC18 cartridges were produced by Supelco (Bellefonte, USA). High-performance liquid chromatography (HPLC) solvent was degassed and filtered through 0.45 μm HA filters (Millipore, Milford, USA). Tissue radioactivity was determined with a calibrated gamma counter (LKB-Wallac Compugamma 1282 CS, Turku, Finland). Ex vivo storage phosphor imaging was performed using a multi-purpose screen (MP, Canberra-Packard Benelux, Groningen, The Netherlands). The exposed screens were scanned with a Cyclone™ storage phosphor system (Packard Biosciences, Meriden, USA).

2.1. Synthesis of 4-(2'-methoxyphenyl)-1-[2'-(*N*-2"-pyridinyl)-*p*-¹⁸F]fluorobenzamido]ethyl-piperazine, [¹⁸F]MPPF

No carrier added [¹⁸F]MPPF was synthesized according to a literature procedure with several adjustments (Le Bars et al., 1998). Aqueous [¹⁸F]fluoride (1 ml) was prepared by the ¹⁸O(*p,n*)¹⁸F nuclear reaction in a 0.8-ml silver target. The enriched water was recovered by elution over an AG1-X8 ion exchange column (K₂CO₃-form). [¹⁸F]Fluoride was eluted with a solution of K₂CO₃ (5 mg) in water (1 ml) into a vial containing Kryptofix 2.2.2. ([K/222] (12–13 mg). The resulting [K/222]⁺¹⁸F[–] complex was dried by four successive azeotropical evaporations with acetonitrile (1 × 1 ml, 3 × 0.5 ml). The precursor, 4-(2'-methoxyphenyl)-1-[2'-(*N*-2"-pyridinyl)-*p*-nitrobenzamido]ethylpiperazine (7 mg) in DMF (0.7 ml), was added and the reaction mixture was heated to 140°C for 25 min. The reaction was quenched with water (4 ml) and the mixture was passed through a Supelclean LC18 cartridge. The cartridge was washed with water (2 ml), 18% EtOH in water (4 ml) and eluted with a mixture of methanol (1 ml) and THF (0.33 ml), into a vial containing 1-ml water. Next, the solution was filtered (0.45 μm, Millex, non-sterile) and purified by HPLC (Phenomenex: Ultracarb ODS 30: 250 × 10 mm; MeOH/THF/NaOAc (0.01 M,

pH = 7.3), 41:14:45; 3.0 ml/min). The fraction containing [^{18}F]MPPF ($t_{\text{R}} = 30\text{--}35$ min, U.V. 254 nm, S.A. > 110 TBq/mmol) was collected, and the product was formulated according to a modified literature procedure (Lemaire et al., 1995). The fraction containing [^{18}F]MPPF was diluted in 100-ml distilled water. Methanol and THF were removed by passing the mixture over a C18 SepPak light cartridge, followed by rinsing of the cartridge with 2×8 ml of 0.9% saline solution. [^{18}F]MPPF was eluted and formulated by passing 0.8 ml ethanol through the cartridge, followed by 8 ml phosphate buffer (pH = 7.4–7.6). After filtration through a 0.22- μm sterile filter (Millex, GP), the product was ready for injection. Quality control using reverse phase HPLC (WATERS NovaPak: 150×3.6 mm, ACN/THF/NaOAc (0.01 M, pH = 5), 28:6:65; 1.5 ml/min: $t_{\text{R}}(\text{MPPF}) = 5$ min, $t_{\text{R}}(\text{MPPNO}_2) = 7$ min) showed a radiochemical purity > 99%. The amounts of WAY 100634 and MPPNO₂ were $\ll 1$ mg/l. The radiochemical yield was 7% (end of bombardment). Total synthesis time was 120 min.

2.1.1. Octanol / water partition coefficient ($\log P$)

1.85–3.7 MBq of [^{18}F]MPPF was dissolved in 0.1 M phosphate buffer pH 7.4 (5 ml), whereafter *n*-octanol (5 ml) was added. The mixture was vigorously shaken at 37°C for 30 min. One hundred microliters of the *n*-octanol layer and of the water layer were counted in a calibrated gamma counter. The partition coefficient was calculated using: $\log P = \log(\text{counts in octanol}/\text{counts in water})$.

2.2. Distribution studies in rat brain

2.2.1. Biodistribution

All experiments were carried out in compliance with the Law on Animal Experimentation of the Netherlands. Male Wistar rats (229 ± 22 g) supplied by Harlan (Lelystad, The Netherlands) were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). Two minutes before administration of [^{18}F]MPPF (2 MBq, S.A. > 110 TBq/mmol), the rats were treated either with saline (control group) or with the 5-HT_{1A} receptor antagonist NAN-190 (2.5 mg/kg) by i.v. injection into the tail vein. One

hour after injection of the radioligand, the animals were sacrificed and 13 areas of the brain were dissected. The time course of the experiment is illustrated in Scheme 2. Tissue radioactivity was measured with a calibrated gamma counter. The samples were weighed and tissue uptake was expressed as standard uptake value (suv): (cpm measured/g tissue)/(cpm injected/g body weight). Residual uptake in the presence of NAN-190 was considered to represent nonspecific binding (Glennon et al., 1988; Rydelek-Fitzgerald et al., 1990). To modulate P-glycoprotein function in control and 5-HT_{1A} blocked animals, cyclosporin A (50 mg/kg) was injected into the penile vein 30 min prior to radioligand administration (Scheme 2).

2.2.2. Dissection of brain regions

The entorhinal cortex and the piriform cortex containing the amygdaloid nucleus were removed on the ventral side of the brain. Olfactory bulbs were dissected and the brain was cut in three coronal slices. The first coronal cut was made ± 2 mm caudal to the olfactory bulbs (A3000 mm), the second just caudal to the optic chiasm (P1500 mm) (Doze et al., 1998; Palkovits and Brownstein, 1988). From the first slice, anterior cingulate/frontopolar cortex was dissected. The second slice was used to isolate striatum and frontal cortex. Thalami could be scooped out from the third brain slice. In the same slice, the remainder of the cortex was peeled off, containing parietal, temporal and occipital areas, after which the hippocampi could be removed as well. Then, the cerebellar hemispheres were removed from the hindbrain and the pons and medulla were obtained by sectioning through the pons just caudal to the occipital cortex. Right and left samples of all brain regions were pooled.

2.2.3. Phosphor screen imaging

Male Wistar rats were either treated with saline (controls), the selective 5-HT_{1A} receptor antagonist NAN-190, or the selective 5-HT_{1A} agonist 8-OH-DPAT, 2 min before injection of 10–15 MBq of [^{18}F]MPPF into the tail vein. After 60 min, the animals were sacrificed. Brains were removed from the skull, frozen on dry ice, and cut into

Scheme 2. Experimental time course of [^{18}F]MPPF distribution studies in rats. P_{CsA} = time of pentobarbital administration in case of cyclosporin A pretreatment, P_{con} = time of pentobarbital administration in experiments without cyclosporin A pretreatment, S/N = saline or NAN-190, R = radioligand, T = termination followed by dissection of tissues.

Fig. 1. Central uptake of [^{18}F]MPPF (60 min p.i.). Specific binding was defined by residual uptake after predosing with NAN-190 (2.5 mg/kg). * Indicates significant difference (Student's *t*-test, $P < 0.05$). CER—cerebellum, THA—thalamus, STR—striatum, MED—medulla, OLF—olfactory bulbs, PTOC—parietal-/temporal-/occipital cortex, FC—frontal cortex, CC—cingulate cortex, APC—amygdala-/piriform cortex, EC—entorhinal cortex, HIP—hippocampus.

slices of 80 μm by means of a microtome (-6 to -10°C). Bregma levels: 2.70, 2.20, 1.60–1.20, 0.20, -1.30 , -1.80 , $-2.30/-2.80$, $-3.60/-3.80$, -4.52 , $-5.20/-5.30$, $-5.60/-5.80$, $-6.04/-6.30$ (Paxinos and Watson, 1998). Three slices were taken from the cerebellum/medulla. All slices were covered with a multi-purpose storage phosphor screen. After 20–24 h (> 10 half-lives) of exposure, the screens were scanned using a storage phosphor system.

2.2.4. Plasma clearance of [^{18}F]MPPF

One of the carotid arteries of anesthetized male Wistar rats (either untreated or treated with Cyclosporin A) was cannulated. After radioligand administration, blood samples (150 μl) were drawn at different time intervals over a

period of 60 min. After centrifugation (Hettich mikroliter; 3 min at 15.000 rpm), plasma radioactivity was measured with a calibrated gamma counter. The samples were weighed and plasma uptake was expressed as standard uptake value. The area under the curve (AUC) was calculated by nonlinear curve fitting.

2.3. Cerebral distribution in wild type and *mdr1a* ($-/-$) knockout mice

Male wild type (27.4 ± 2 g, $n = 5$; Harlan, The Netherlands) or *mdr1a* ($-/-$) FVB mice (31.3 ± 2 g, $n = 3$; kindly provided by The Netherlands Cancer Institute in Amsterdam) between 9 and 16 weeks of age were anes-

Fig. 2. Ex vivo autoradiography of rat brain at different levels using phosphor storage imaging. Top images (A) represent control slices, bottom images (B) represent the corresponding slices after pretreatment with NAN-190 (2.5 mg/kg). I—Septal area, II—Dentate gyrus, III/IV—Hippocampus.

Fig. 3. Comparison of the tissue/cerebellum ratios of [^{18}F]MPPF obtained from ex vivo autoradiography with regional 5-HT $_{1A}$ receptor densities determined by in vitro autoradiography with [methoxy- ^3H]WAY 100635 (Khawaja, 1995). (■)—Caudate Nucleus, (▽)—Frontal cortex, (●)—Septo hippocampal area, (◇)—Cingulate cortex, (⊗)—Amygdala hippocampal area, (△)—Lateral septum ventral, (⊕)—Hippocampus CA1, (□)—Dentate gyrus, (◆)—Lateral septum dorsal, (○)—Lateral septum intermediate, (▲)—Hippocampus CA3.

thetized by intraperitoneal injection of ketamine/xylazine (Ketalar®/Rompun®; 2:1, 1 ml/kg). One hour after i.v. administration of [^{18}F]MPPF into the tail vein, the animals were sacrificed and several tissues were dissected according to the procedure mentioned above. Tissue uptake of radioactivity was expressed as standard uptake value.

3. Results

3.1. [^{18}F]MPPF uptake and distribution in rat brain

We measured a $\log P$ of 2.14 ± 0.06 ($n = 5$) for [^{18}F]MPPF. On the basis of this value, a good uptake of the radioligand in the brain could be expected. However, measured uptake values in biodistribution experiments were quite low. The uptake of [^{18}F]MPPF in the brain of saline- and NAN-190 pretreated Wistar rats is presented in Fig. 1. Standard uptake values were in the range of 0.05–0.25. A significant reduction caused by NAN-190 (2.5 mg/kg) was observed in hippocampus, amygdala/piriform-, cingulate-/frontopolar-, and entorhinal cortex (Student's t -test, $n = 6$; $P < 0.05$).

Because of the low standard uptake values, ex vivo phosphor storage imaging experiments were performed to confirm the in vivo selectivity of [^{18}F]MPPF for the 5-HT $_{1A}$ receptor in rat brain. The results for saline- and NAN-190 pretreated rats are displayed in Fig. 2. Uptake was highest in septal and hippocampal areas (Fig. 2A; $n = 3$). After treatment with NAN-190 (2.5 mg/kg; $n = 3$), specific binding was no longer observed (Fig. 2B).

Tissue/cerebellum ratios for [^{18}F]MPPF in the phosphor storage imaging experiments were excellently correlated with receptor densities measured by in vitro autoradiography in the same animal species using [methoxy- ^3H]WAY 100635 (Fig. 3) (Khawaja, 1995)].

Fig. 4. Central uptake of [^{18}F]MPPF (60 min p.i.) after pretreatment with cyclosporin A (50 mg/kg) 30 min prior to injection of the radioligand. Specific binding and regions are defined as in Fig. 1.

Fig. 5. Comparison of the tissue/cerebellum ratios of [¹⁸F]MPPF in vivo after modulation with cyclosporin A (50 mg/kg) with regional 5-HT_{1A} receptor densities determined by ex vivo autoradiography (Hume et al., 1994). (1)—Striatum, (2)—Thalamus, (3)—Medulla, (4)—Olfactory bulbs, (5)—Frontal cortex, (6)—Amygdala/piriform cortex, (7)—Cingulate/frontopolar cortex, (8)—Entorhinal cortex, (9)—Hippocampus.

3.2. Influence of P-glycoprotein modulation on central uptake and distribution of [¹⁸F]MPPF in rats

After modulation of P-glycoprotein functionality by pretreatment of animals with cyclosporin A (50 mg/kg, 30

min prior to administration of [¹⁸F]MPPF), tissue uptake was increased in all brain regions (Fig. 4, compare Fig. 4 with Fig. 1; $P < 0.05$). A significant reduction of tissue radioactivity by NAN-190 (2.5 mg/kg) could be seen in hippocampus, amygdala/piriform-, cingulate/frontopolar-,

Fig. 6. Difference in cerebral uptake between FVB wild and *mdr1a*(-/-) knockout mice. * Indicates significant difference (Student's *t*-test, $P < 0.05$).

entorhinal cortex and also in the frontal- and in the parietal-/temporal-/occipital cortex (Fig. 4).

In Fig. 5, tissue/cerebellum ratios of [^{18}F]MPPF in cyclosporin A-treated animals are compared to receptor density values obtained from biodistribution studies with [methoxy- ^3H]WAY 100635 (Hume et al., 1994). A good correlation is observed and the regression line intercepts the Y-axis at approximately $Y = 1$ (a receptor density of zero corresponds to a tissue/cerebellum ratio of 1).

Because the levels of radioactivity in plasma were increased after 60 min in the cyclosporin A-treated animals, the effect of cyclosporin A on ligand delivery to the brain was determined. The clearance rate of injected radioactivity was determined by analyzing the AUC for control and cyclosporin A-treated animals. A significant decrease in the clearance of MPPF-derived radioactivity was observed after treatment with cyclosporin A, leading to a 1.84 ± 0.37 -fold increase of the AUC ($n = 5$ for control, $n = 5$ for cyclosporin A-treated animals).

3.3. Comparison between brain uptake of [^{18}F]MPPF in FVB wild-type and *mdr1a*(–/–) knockout mice

To confirm that the increased uptake of [^{18}F]MPPF in rat brain after pretreatment with cyclosporin A is due to modulation of P-glycoprotein functionality, biodistribution was compared in FVB-wild ($n = 5$) and *mdr1a*(–/–) knockout mice ($n = 3$). Uptake of [^{18}F]MPPF in the brain of *mdr1a*(–/–) knockout mice was two- to three-fold higher than in the wild type (Fig. 6).

4. Discussion

Biodistribution experiments showed a five- to seven-fold lower brain uptake for the 5-HT_{1A} receptor antagonist MPPF compared to the prototype ligand WAY 100635 (Fig. 1A) (Plenevaux et al., 1997; Shiue et al., 1997; Pike et al., 1994). This was unexpected since the lipophilicity of MPPF is sufficient for rapid diffusion through the blood–brain barrier ($\log P = 2.14$). Since biodistribution is a rather crude method that does not provide information on ligand uptake in small (greater than a few millimeter) areas, ex vivo autoradiography was performed using a phosphor storage system (Fig. 2). A laminar distribution of radioactivity was observed, which corresponds to 5-HT_{1A} receptor distribution in rat brain (Fig. 2A). Tissue/cerebellum ratios obtained from these images closely correlated with known receptor densities as determined by in vitro autoradiography (Fig. 3) (Khawaja, 1995). Pretreatment with NAN-190, a selective 5-HT_{1A} partial receptor antagonist (Glennon et al., 1988) and with 8-OH DPAT (data not shown), a selective 5-HT_{1A} receptor agonist (Arvidsson et al., 1981; Rydelek-Fitzgerald et al., 1990), reduced the uptake of [^{18}F]MPPF to the same values as were observed for cerebellum (Fig. 2B), confirming that [^{18}F]MPPF binds selectively to 5-HT_{1A} receptors in rat brain in vivo.

To determine the cause of the reduced cerebral uptake of MPPF as compared to WAY 100635, we decided to test if the former is a substrate for P-glycoprotein. After treatment with cyclosporin A, brain uptake of [^{18}F]MPPF was increased four- to six-fold (Figs. 1 and 4). Whereas in control rats, no significant specific binding could be observed in the frontal-, parietal-, temporal-, and occipital cortices in the biodistribution experiments (Fig. 1), modulation of P-glycoprotein functionality with cyclosporin A led to a significant difference between control and NAN-190-pretreated animals in these regions (Fig. 4), while tissue/cerebellum ratios remained in good agreement with receptor densities as determined with [methoxy- ^3H]WAY 100635 (Fig. 5) (Hume et al., 1994). It is clear that in some brain areas, P-glycoprotein modulation results in increased radioligand uptake and, therefore, improved counting statistics.

The increase of [^{18}F]MPPF derived radioactivity in the brain after administration of cyclosporin A was partially due to increased ligand delivery to the brain (AUC in plasma: cyclosporin A/control = 1.84). To prove that [^{18}F]MPPF is a substrate for P-glycoprotein and that the observed differences in cerebral uptake between cyclosporin A-treated and control animals were not solely due to altered pharmacokinetics, we compared the cerebral uptake in wild type and *mdr1a*(–/–) knockout mice. A significant increase (two- to three-fold) was found in the knockout mice (Fig. 6). These results lead us to conclude that the extra aromatic ring in MPPF causes an increased efflux action exerted by P-glycoprotein, and a lower brain uptake than would be predicted from its lipophilicity. The excellent results obtained with volunteers and with ex vivo storage phosphor imaging of rat brain show that the interaction with P-glycoprotein is not associated with a diminished selectivity of [^{18}F]MPPF for the 5-HT_{1A} receptor (Passchier et al., 2000a,b), but it does stress the importance of taking the presence of P-glycoprotein into account during the development of radioligands for CNS receptors and for 5-HT_{1A} receptor antagonists in particular.

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