

Effect of endogenous serotonin on the binding of the 5-HT_{1A} PET ligand ¹⁸F-MPPF in the rat hippocampus: kinetic β measurements combined with microdialysis

Luc Zimmer,⁽¹⁾ Gweltas Mauger,⁽¹⁾ Didier Le Bars,⁽¹⁾ Gregory Bonmarchand,⁽¹⁾ Andre Luxen⁽²⁾ and Jean-Francois Pujol⁽¹⁾

⁽¹⁾CERMEP Biomedical Cyclotron, Lyon, France

⁽²⁾Cyclotron Research Center, Liège University, Liège, Belgium

Abstract

By using a combination of an original β^+ -sensitive intracerebral probe and microdialysis, the effect of increased endogenous serotonin on specific binding of ¹⁸F-MPPF [4-(2'-methoxy-phenyl)-1-[2'-(N-(2"-pyridinyl)-p-fluorobenzamido)ethyl]piperazine] to the serotonin-1A (5-HT_{1A}) receptors was investigated in the hippocampus of the anaesthetized rat. Our β -sensitive probe prototype was sensitive enough to obtain specific ¹⁸F-MPPF time-activity curves in the rodent (hippocampus/cerebellum ratio ≈ 2). The serotonin neuronal release was pharmacologically enhanced using fenfluramine at three different doses (1, 2 and 10 mg/kg intravenous) multiplying by 2-15 the extracellular serotonin in the hippocampus. These extracellular variations of extracellular serotonin resulted in dose-ranging decreases in ¹⁸F-MPPF-specific binding in the same rat. Our results showed for the first time that ¹⁸F-MPPF binding could be modulated by modifications of extracellular serotonin in the rat hippocampus. These results were confirmed by the enhancement of extracellular radioactivity collected in dialysates after the displacement of ¹⁸F-MPPF by fenfluramine. After modelization, ¹⁸F-MPPF binding could constitute an interesting radiotracer for positron emission tomography in evaluating the serotonin endogenous levels in limbic areas of the human brain.

Keywords: β microprobe, fenfluramine, 5-HT_{1A} receptors, microdialysis, MPPF, serotonin.

During the last decade, it has become clear that serotonin (5-HT) is a central neurotransmitter and neuromodulator involved in many physiological functions and pathological disorders. The serotonin-1A (5-HT_{1A}) receptors play an important role in various physiological processes, including the regulation of mood, sleep and sexual behaviour (Hillver *et al.* 1990; Gardner *et al.* 1997; Seifritz *et al.* 1997), as well as learning (Meneses and Hong 1997; Meneses 1999) or psychiatric disorders, such as anxiety, depression and schizophrenia (Kostowski *et al.* 1989; Glennon 1990; Saudou and Hen 1994; Peroutka 1995; Saxena 1995).

Positron emission tomography (PET) has the unique ability to quantitatively monitor physiological changes in the living brain. Several radioligands have been developed for the imaging and quantification of 5-HT_{1A} receptors with PET, and several of these have been tested in humans. In particular, [carbonyl-¹¹C] (N-2-(4-(2-methoxyphenyl-l-piperazinyl)ethyl)-N-(2-pyridil)-cyclohexanecarboxamide ([carbonyl-¹¹C]WAY 100635) has been reported to bind to the 5-HT_{1A} receptors [inhibition constant (K_i) = 0.8 nmol/L, Zhuang *et al.* 1994].

Recently, the selective 5-HT_{1A} antagonist 4-2'-(methoxy-phenyl)-1-[2'-(N-2"-pyridinyl)-p-fluorobenzamido]ethylpiperazine (MPPF) has successfully been labelled with ¹⁸F-fluorine, resulting in the ¹⁸F-fluoro analogue ¹⁸F-MPPF (Shiue *et al.* 1997). Animal experiments have shown a regional distribution of this radioligand that concurs well with known 5-HT_{1A} receptor densities (Hamon *et al.* 1990), with a high uptake in the hippocampus and a low uptake in the receptor-poor cerebellum (Shiue *et al.* 1997; Le Bars *et al.* 1998; Ginovart *et al.* 2000; Plenevaux *et al.* 2000b).

It is known that ¹⁸F-MPPF has a relatively low affinity for the 5-HT_{1A} receptor (K_i = 3.3 nmol/L) (Zhuang *et al.* 1994). Moreover, the relative affinity of 5-HT for the 5-HT_{1A} receptors is K_i = 4.17 nM (Van Wijngaarden *et al.* 1990), and ¹⁸F-MPPF may therefore be more suitable for the detection of changes in endogenous 5-HT. While the affinity characteristics of ¹⁸F-MPPF are well-known, to our knowledge no data are available in the literature documenting the displacement of this radioligand by endogenous 5-HT. Therefore, the aim of our study was to demonstrate that ¹⁸F-MPPF could be displaced by endogenous 5-HT in the rat hippocampus.

We adopted an original approach using the prototype of a new detector dedicated to the measurement of the kinetics of PET radioligands in the rat brain (Pain *et al.* 2000; Zimmer *et al.* 2002). This consists of a p-sensitive intracerebral probe (SIC, French acronym for: 'sonde intracérébrale') stereotaxically implanted in the hippocampus and allowing local counting of radioactivity. This approach was coupled with microdialysis which allows the measurement of the extracellular 5-HT. To assess the validity of the displacement of ¹⁸F-MPPF by endogenous 5-HT, the following investigations were conducted: (i) we verified the ability of SIC to measure the ¹⁸F-MPPF specific binding; (ii) we studied the relationship between changes in 5-HT concentration (measured by microdialysis) and changes in binding parameters of ¹⁸F-MPPF (measured with SIC); (iii) we validated our

results by the measurement of the extracellular radioactivity release (measured by microdialysis). The main prospect of this study is the measurement of changes in 5-HT levels in the human brain using PET.

Abbreviations used: 5-HT, 5-hydroxytryptamine or serotonin; SIC, 'sonde intracérébrale' or β-microprobe; MPPF, 4-(2'-methoxyphenyl)-l-[2'-[N-(2"-pyridmethyl)-p-fluorobenzamido]ethyl]piperazine; PET, positron emission tomography; WAY-100635, 4-(2'-methoxyphenyl)-l-[2'-[N-(2"-pyridinyl)cyclohexylcarboxamido]ethyl]piperazine.

Materials and methods

Synthesis of ¹⁸F-MPPF

¹⁸F-MPPF was synthesized with a radiochemical yield of 25% (decay corrected) in an automated system (Le Bars *et al* 2001), using the chemical pathway previously described (Le Bars *et al* 1998). Chemical and radiochemical purity were higher than 98% as determined by HPLC. Specific activity from the injected radiotracer ranged from 37×10^3 MBq/μmol to 111×10^3 MBq/μmol (1-3 Ci/μmol).

SIC components

SIC apparatus is a β-sensitive microprobe stereotactically implanted in the rat brain. This prototype was designed and manufactured in the Institut de Physique Nucléaire (CNRS, Orsay, France). Briefly, the sensitive end of the probe consists of a 1-mm long and 1-mm diameter plastic scintillating fibre (Bicron BCF-12). SIC takes advantage of the short range of β particles within biological tissues, which limits the detection volume surrounding the probe. The positron energy distribution of fluorine-18 and its linear energy loss therefore leads to a 1.1-mm maximum range in biological tissue for the emitted β particles (Pain *et al* 2000). This detection tip is fused to a 1-mm diameter optic fibre (Bicron BCF-98) whose length may be adjusted. The optic fibre is coupled to a photomultiplier (R7400P, Hamamatsu) characterized by its small size, very low thermal noise and high amplification gain. In addition to the photomultiplier, this version of SIC is composed of an interface module ensuring the readout of the photomultiplier signal through an amplifier integrator and a programmable threshold discriminator. The final PC software used is Lab View® which controls and generates the acquisition. It allows the user to adjust voltage and threshold for each photomultiplier and to set the integration time (from 1 s to 5 min).

SIC implantation coupled to the microdialysis

Male Sprague-Dawley rats (IFFA Credo) weighing 300-400 g were housed under standard conditions of temperature and humidity in artificial light (light from 08.00 to 20.00 h). All experimental procedures were in compliance with EEC guidelines and directives (86/09/EEC). A total of 27 male rats were used in this study. The rats were anaesthetized by a single intraperitoneal injection of urethane (Sigma Aldrich, Lyon, France) at a dose of 1.7 g/kg body weight and remained anaesthetized throughout all procedures. Following catheterization of a tail vein, the rats were positioned on a stereotaxic apparatus (LPC). The skull was exposed and the bregma point visualized. One SIC probe was implanted in the hippocampus, and the second in the cerebellum (Fig. 1). The coordinates of implantation were: A/P -5.0, L/M 5.0, and V/D -8.0 (hippocampus); A/P -12.0, L/M 3.0, and V/D -4.0 (cerebellum), from the bregma point and the dura, respectively, according to the atlas of Paxinos and Watson (1986). A microdialysis probe (polycarbonate, 15 kDaA cutoff, 3 mm length, MAB 6.20.3) was implanted in the same animal in the hippocampal contralateral side (A/P-5.0, L/M -5.0, and V/D -8.0).

The probe was immediately and continuously perfused with perfusion buffer (Dulbecco's modified liquid, ICN + 2.2 mmol/L CaCl₂) at 1.0 μL/min using a microsyringe pump (Harvard Instruments, South Natick, MA, USA). Body temperature was maintained at $37 \pm 1^\circ\text{C}$ throughout the test period using a thermostatically controlled heating blanket (CMA/Microdialysis).

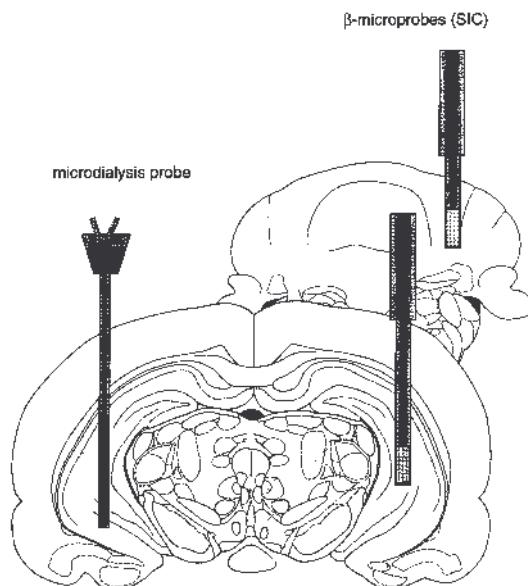


Fig. 1 Locations of the β -sensitive probes (SIC) in the hippocampus and the cerebellum and location of the microdialysis probe in the contralateral hippocampus of the anaesthetized rat.

SIC scanning and microdialysis procedures

SIC acquisitions and dialysate collections were carried out 2 h after implantation of the probes. This time corresponds with the neurotransmission stabilization period (Benveniste 1989). Concerning the SIC implantation, this time was used since our pilot studies showed that the ^{18}F -MPPF binding characteristics were not modified when the radioligand was injected at 2, 4 or 6 h post-implantation. For each SIC acquisition, 74 MBq (2 mCi) ^{18}F -MPPF (in a volume of 0.4 mL saline) were injected via the tail vein over a 45-s period and the time course of radioactivity was studied for 180 min using 10-s time integration acquisition. During this time, the microdialysis flow rate was maintained at 1 $\mu\text{L}/\text{min}$.

In a first group of rats, each rat had a 2-mCi ^{18}F -MPPF injection. The ^{18}F -MPPF binding was measured with SIC in the hippocampus and the cerebellum. Simultaneously, dialysates were collected in the hippocampus every 10 min and the extracellular 5-HT was measured with electrochemical HPLC.

In a second group, each rat had a 74 MBq (2 mCi) ^{18}F -MPPF injection followed at 20 min by a fenfluramine injection, which is a 5-HT releaser, at three different pharmacological doses (1 mg/kg, 2 mg/kg and 10 mg/kg). The ^{18}F -MPPF binding was measured with SIC in the hippocampus and the cerebellum. Simultaneously, dialysates were collected in the hippocampus every 10 min and the extracellular 5-HT was measured with electrochemical HPLC.

In a third group, each rat had a 74 MBq (2 mCi) ^{18}F -MPPF injection followed at 30 min by a WAY 100635 injection [5 mg/kg intravenously (i.v.) in saline], which is a specific 5-HT_{1A} receptor antagonist (4-(2'-methoxyphenyl)-1-[2'-(N-(2"-pyridinyl)cyclo-hexylcarboxamido)-ethyl]piperazine, synthesized by Liege CRC group). The ^{18}F -MPPF binding was measured with SIC in the hippocampus and the cerebellum.

In a fourth group, the rats were implanted with two microdialysis probes (one in the hippocampus and one in the cerebellum, according to the previous coordinates). Each rat had a 74 MBq (2 mCi) ^{18}F -MPPF injection followed at 30 min by a 10-mg/kg fenfluramine injection. Dialysates were collected every 5 min in both areas and the radioactivity of the dialysates was measured using an automated gamma-counter (Cobra II; Packard, Meriden, CT, USA) calibrated in the fluorine-18 energy range.

HPLC analysis of hippocampal dialysates

The dialysate level of 5-HT was measured using HPLC with electrochemical detection, directly after *in vivo* collection. Briefly, the isocratic mobile phase was composed of 75 mM NaH₂PO₄, 0.1 mM EDTA, 0.3 mM OSA + 18% methanol (pH 4.3). This mobile phase was pumped at 0.4 mL/min with a Lachrom system (Merck-Hitachi, Darmstadt, Germany). Separation was performed with a column C18 reverse phase (Uptisphere ODB, 3 μm , 100 x 3 mm). The electrochemical detector (Antec-Leyden, Zoeterwoude, the Netherlands) was composed of a glassy carbon working set at + 0.6 V with reference to an Ag/AgCl electrode. Signals were recorded and quantified by the Beckman Gold 118 integrator, calibrated with a standard aqueous solution of 5-HT. Under these conditions, the detection limit was 0.5 fmol injected for 5-HT.

Probe placement controls

After protocol acquisition, the anaesthetized rats were killed and their brains quickly removed and frozen at —

80°C. Coronal 20- μ m-thick tissue sections were cut in a cryostat (Microm, Heidelberg, Germany) at —20°C throughout the striatum and the cerebellum, and thaw-mounted onto glass slides. The sections were coloured by Cresyl blue and the probe placements were atlas-matched.

Data analysis

The SIC data (expressed in mean of disintegration per 10 s) were averaged every minute. These data were radioactive-decay corrected and were normalized with the activity injected and the specific radioactivity. Statistical analyses were conducted by comparing the means obtained from the control and treated animals for each time value (every min) by one-way ANOVA with repeated measurements, followed by a post-hoc Student's *t*-test. A *p*-value of less than 0.05 was considered to be statistically significant.

Results

Radioactivity kinetic curves of ¹⁸F-MPPF in the hippocampus and the cerebellum of control rats

In all studies, a significant amount of radioactivity was already accumulated in the hippocampus 10 min after administration of ¹⁸F-MPPF. ¹⁸F-MPPF activity in the hippocampus and cerebellum stabilized 20 min after injection with a ratio hippocampus/cerebellum \approx 2 ($n = 4$ rats). The hippocampal activity decreased slowly within 3 h, whereas the cerebellar activity remained relatively constant (Fig. 2a).

Specific binding of ¹⁸F-MPPF

The MPPF-specific binding calculated by the cerebellum activity deducted from the hippocampus activity averaged 30 Bq, 30 min post-injection and reached 20 Bq, 180 min after the 74 MBq ¹⁸F-MPPF injection (Fig. 2b).

Extracellular radioactivity in the hippocampus and the cerebellum of control rats after ¹⁸F-MPPF injection

In rats implanted with two microdialysis probes (one in the hippocampus and one in the cerebellum, $n = 4$ rats), measurement of the extracellular radioactivity revealed that the gamma radioactivity was higher in the cerebellum than in the hippocampus from 20 min until 40 min after the 74 MBq ¹⁸F-MPPF injection (*p* < 0.05). After this time the two curves were superimposed (Fig. 2c).

5-HT extracellular level in the hippocampus

The electrochemical measurement of the 5-HT in dialysates showed that, during the control experiment, the level of the neurotransmitter in the hippocampus did not significantly change (1.5 ± 0.1 fmol/ μ L, without probe correction, results not shown).

5-HT challenge studies: ¹⁸F-MPPF and fenfluramine injections

To determine if changes in 5-HT levels would alter ¹⁸F-MPPF uptake, the 5-HT releaser fenfluramine was intravenously administered 20 min after the ¹⁸F-MPPF injection, under equilibrium binding conditions, at doses of 1, 2 and 10 mg/kg. At the same time the specific binding of ¹⁸F-MPPF was measured using the SIC β -microprobe. Figure 3(a, upper curve) shows that injection of 1 mg/kg fenfluramine ($n = 4$ rats) induced in the hippocampus a 20% increase of extracellular 5-HT compared to baseline level in control rats. At the same time and on the same rats, the SIC measurements in the contralateral hippocampus revealed that the ¹⁸F-MPPF-specific binding decreased significantly and reproducibly in comparison with the control rats curve (Fig. 3a, lower curve). This 25% decrease (*p* < 0.05) occurred simultaneously with the extracellular 5-HT increase.

Figure 3(b, upper curve) shows that a 2-mg/kg fenfluramine injection ($n = 4$ rats) induced in the hippocampus a 50% increase of extracellular 5-HT in comparison with the control rats curve. At the same time, the SIC curve reveals a 60% decrease (*p* < 0.05) of ¹⁸F-MPPF binding in comparison with the control rats curve (Fig. 3b, lower curve).

When 10 mg/kg fenfluramine was injected ($n = 4$ rats), we observed a pronounced increase of the extracellular 5-HT (a 15-fold increase vs. the control level; Fig. 3c, upper curve). This massive increase was simultaneously accompanied by a total displacement of the ¹⁸F-MPPF-specific binding (*p* < 0.05, Fig. 3c, lower curve). It should be noted that the level of radioactivity in the cerebellum was not affected by the treatment by fenfluramine at 1, 2 or 10 mg/kg.

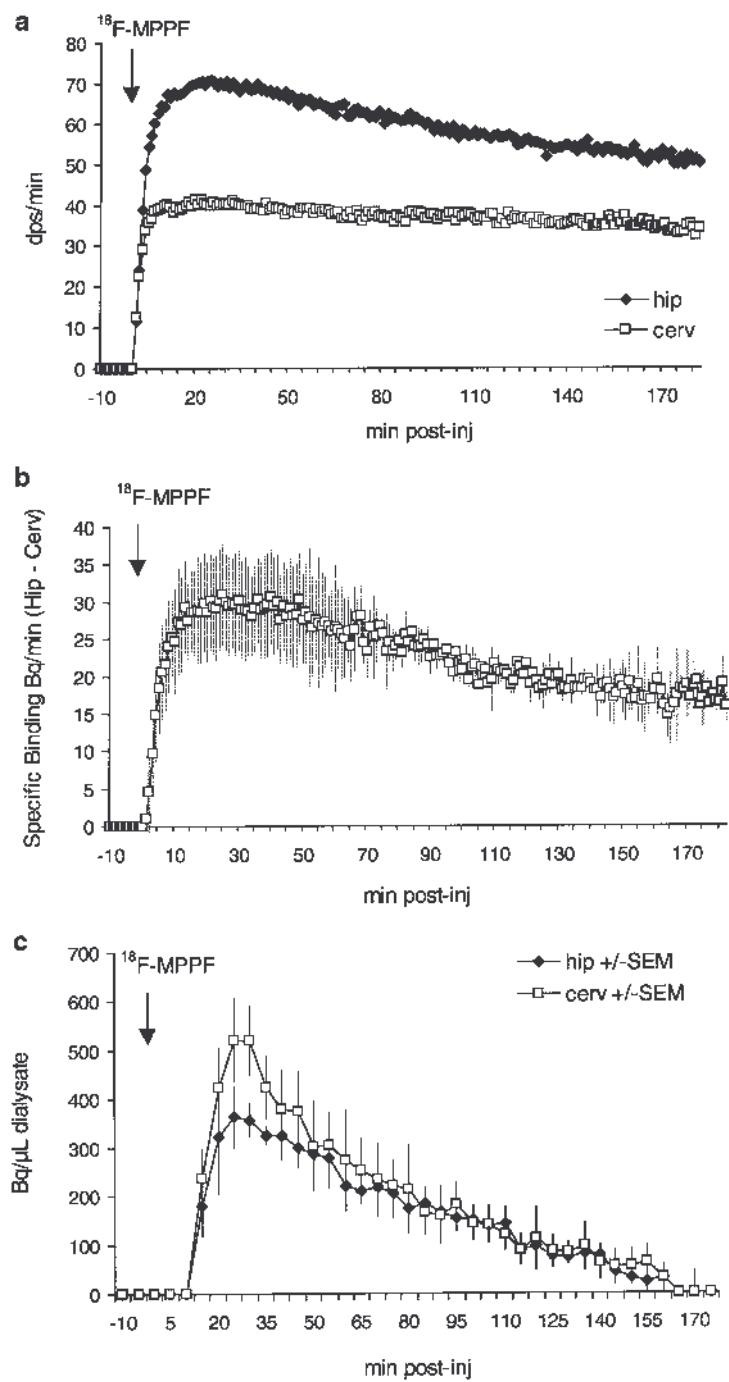


Fig. 2 (a) Radioactivity kinetic curve measured by the β -microprobe SIC in the hippocampus and the cerebellum of control anaesthetized rats after a 74 MBq ^{18}F -MPPF injection (10 s acquisition averaged every min, mean of four rats). The arrow figures the ^{18}F -MPPF injection, (b) Specific binding of ^{18}F -MPPF calculated by the cerebellum activity deducted from the hippocampus activity ($n = 4$ rats \pm SEM). (c) Extracellular radioactivity of the microdialysates measured by an auto-gamma-counter collected every 5 min in the hippocampus and the cerebellum of anaesthetized rats after a 74-MBq ^{18}F -MPPF injection ($n = 4$ rats \pm SEM).

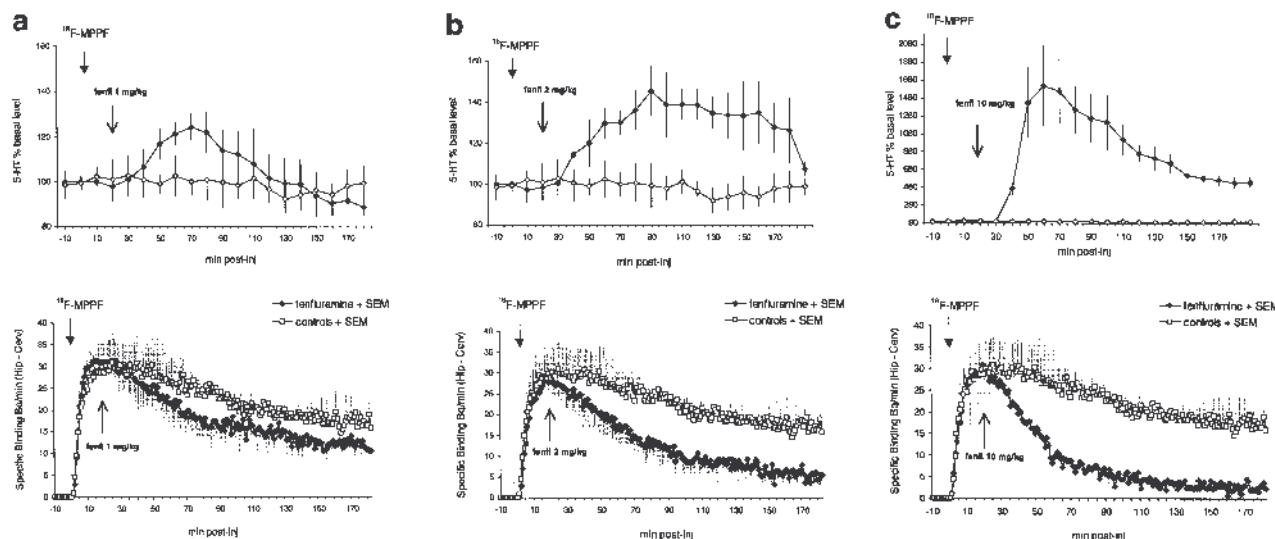


Fig. 3 Upper curves: Variation of extracellular 5-HT in the hippocampus of anaesthetized rats with a 74 MBq ¹⁸F-MPPF injection followed at 20 min by (a) a 1-mg/kg fenfluramine i.v. injection, (b) a 2-mg/kg fenfluramine i.v. injection and (c) a 10-mg/kg fenfluramine i.v. injection (◆, fenfluramine, n = 4 ± SEM for each dose) in comparison with control rats without fenfluramine (□, controls, n = 4 ± SEM). The arrows figure the ¹⁸F-MPPF and the fenfluramine injections. Lower curves: Variation of the specific binding of ¹⁸F-MPPF in the same rats measured by the β-microprobe SIC.

Relation between the ¹⁸F-MPPF-specific binding and the 5-HT extracellular level

The results of the SIC ¹⁸F-MPPF binding dose-response experiments were compared to the microdialysis measurement of fenfluramine-induced 5-HT release (dose range 1-10 mg/kg) by comparing the area under curves (AUC). A linear relationship was observed between fenfluramine-induced 5-HT release and fenfluramine-induced ¹⁸F-MPPF displacement between 0 and 2 mg/kg ($r^2 = 0.99$). Over 2 mg/kg and at 10 mg/kg the fenfluramine dose induced a large increase in 5-HT concentrations which led to a total displacement of the ¹⁸F-MPPF binding.

¹⁸F-MPPF displacement after WAY 100635 injection

Displacement studies were performed to assess the pharmacological specificity of the *in vivo* uptake of ¹⁸F-MPPF (n = 3 rats). After ¹⁸F-MPPF activity stabilized in the hippocampus and the cerebellum (30 min), unlabelled WAY 100635 was administered as an intravenous bolus (5 mg/kg). The specific binding of ¹⁸F-MPPF decreased significantly within 30 min ($p < 0.05$), in comparison with the control rats curve. On average, across three displacement studies, a $90 \pm 10\%$ decrease in specific ¹⁸F-MPPF activity was observed (Fig. 4). It should be noted that the level of radioactivity in the cerebellum was not affected by the treatment by WAY 100635.

Variation of extracellular radioactivity after fenfluramine injection

Figure 5 represents the variation of the extracellular radioactivity of ¹⁸F-MPPF in the hippocampus (Fig. 5a) and the cerebellum (Fig. 5b) in rats having undergone two micro-dialysis probes: one in the cerebellum and one in the hippocampus (n = 4 rats). Within 20 min of the 10-mg fenfluramine injection, more extracellular labelled MPPF was collected in the hippocampus dialysates in comparison with the control rats ($p < 0.05$). One hour after fenfluramine injection, this radioactivity was lower in the fenfluramine-rats than in the control rats ($p < 0.05$). Finally, the total radioactivity collected in dialysates was the same in the control rats and the fenfluramine-rats (Fig. 5a, inset). At the same time the extracellular radioactivity in the cerebellum was unchanged after the fenfluramine injection in comparison with control rats.

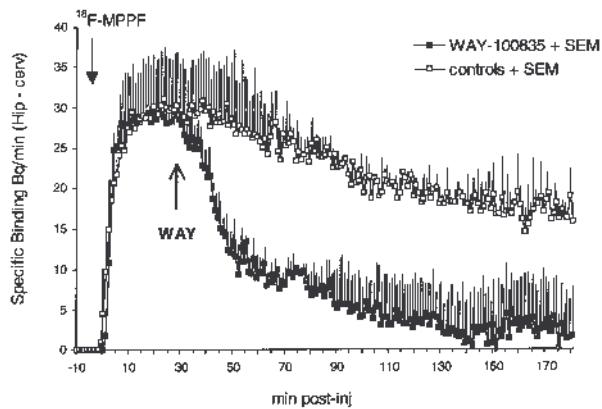


Fig. 4 Variation of the specific binding of ^{18}F -MPPF (74 MBq injected) measured by the β -microprobe SIC after the i.v. injection of 5 mg/kg of WAY 100635 in comparison with control rats (WAY 100635, $n = 3 + \text{SEM}$; controls, $n = 4 + \text{SEM}$). The arrows figure the ^{18}F -MPPF and the WAY 100635 injections.

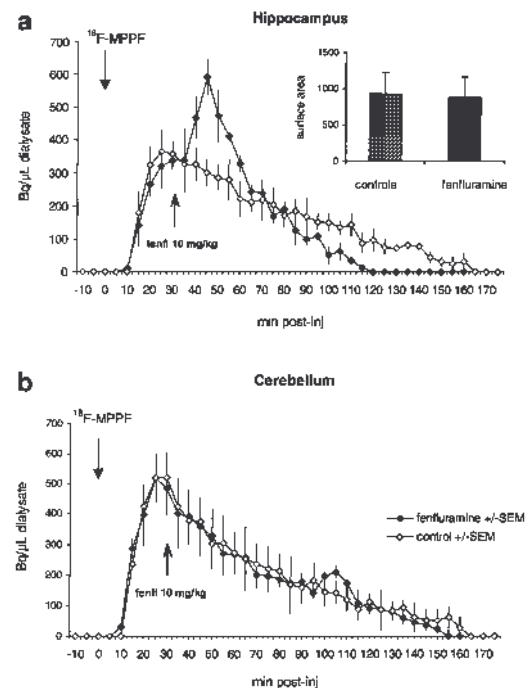


Fig. 5 (a) Extracellular radioactivity measured by an auto-gamma-counter of the microdialysates collected every 5 min in the hippocampus of anaesthetized rats after a 74 MBq ^{18}F -MPPF i.v. injection followed by a 10-mg/kg fenfluramine injection (\blacklozenge , fenfluramine, $n = 4$ rats $\pm \text{SEM}$) in comparison with control rats (\lozenge , controls, $n = 4$ rats $\pm \text{SEM}$). The arrows figure the ^{18}F -MPPF and the fenfluramine injections. Inset: Surface area (in arbitrary units $\pm \text{SEM}$) representing the total radioactivity collected in the dialysates after ^{18}F -MPPF injection in fenfluramine-treated or in control rats, (b) Extracellular radioactivity measured by an auto-gamma-counter of the microdialysates collected every 5 min in the cerebellum of the same anaesthetized rats after a 74 MBq ^{18}F -MPPF i.v. injection followed by a 10-mg/kg fenfluramine injection (\blacklozenge , fenfluramine, $n = 4$ rats $\pm \text{SEM}$) in comparison with control rats (\lozenge , controls, $n = 4$ rats $\pm \text{SEM}$).

Discussion

Recently, several radiotracers have been developed for the imaging and quantification of 5-HT_{1A} receptors with PET (Passchier and van Waarde 2001). The selective 5-HT_{1A} antagonist MPPF has successfully been labelled with fluorine-18, resulting in fluoro-MPPF (Shiue *et al* 1997; Le Bars *et al* 1998). ^{18}F -MPPF is a well-known 5-HT_{1A} radioligand for 5-HT_{1A} imaging. Pharmacological experiments have shown that MPPF is an antagonist of the pre and postsynaptic 5-HT_{1A} receptors (Zhuang *et al* 1994; Thielen *et al* 1996) and that MPPF presents a

high selectivity for this receptor (Kung *et al* 1996). Radiopharmacological experiments have demonstrated that the fluorine-labelled MPPF presents an important initial uptake in the brain and that there are few radioactive metabolites in the brain (Plenevaux *et al* 2000b). 5-HT_{1A} receptors are concentrated mainly in limbic areas, notably in the hippocampus (Kia *et al* 1996). The autoradiographies of ¹⁸F-MPPF are consistent with the known distribution of 5-HT_{1A} receptors (Palacios *et al* 1990) with a high labelling in the hippocampus and low labelling in the cerebellum, which is poor in 5-HT_{1A} receptors (Plenevaux *et al* 2000a,b). Moreover, it is known that ¹⁸F-MPPF has a relatively low affinity for the 5-HT_{1A} receptor ($K_i = 3.3$ nM, Zhuang *et al* 1994) and that the relative affinity of 5-HT for the 5-HT_{1A} receptors is comparable ($K_i = 4.17$ nM, Van Wijngaarden *et al* 1990). Our hypothesis was therefore that ¹⁸F-MPPF-specific binding may be influenced by the changes in cerebral 5-HT concentrations.

With a view to demonstrating our hypothesis, we used a prototype of a new detector dedicated to the measurement of kinetic radiotracer in a small animal brain area ('SIC'). This was done on the basis of a small β-range-sensitive scintillating probe coupled to an ultra-compact and low-noise photomultiplier tube (Pain *et al* 2000). The detector takes advantage of the limited range of β particles within biological tissues to determine a limited 'detectable thickness' surrounding the probe. *In vitro* calibrations showed that for fluorine-18-labelled radiotracers, 90% of the count rate measured by the probe corresponds to β⁺ emitted at a distance of 1.1 mm from the tip of the probe (Pain *et al* 2000). Most particles emitted from a distance greater than 1.1 mm from the probe will be stopped in tissue and will not be detected. The probe sensitivity was experimentally determined to be 0.55 cp/KBq/mL for fluorine-18 (Pain *et al* 2000). In a previous study, we demonstrated that the sensitivity and selectivity of the device make it suitable for use in the study of the kinetic properties of established or potential PET ligands in small animals (Zimmer *et al* 2002).

In this study, the SIC β-microprobe, stereotactically implanted in the hippocampus and the cerebellum, makes it possible to count the MPPF radioactivity locally. In our study, it was assumed that there were no regional differences in radioligand delivery and non-specific binding (Ichise *et al*. 2001). According to previous ¹⁸F-MPPF studies (Passchier *et al*. 2000a,b), the cerebellum was used as reference tissue because this region is practically devoid of 5-HT_{1A} receptors (Pazos *et al*. 1987; Burnet *et al*. 1995). Thus the specific binding was estimated as the difference between the concentration of radioligand in the reference region (cerebellum, non-specific) and in the region of interest (hippocampus, specific + non-specific). This approach was coupled with microdialysis which allows the measurement of the extracellular 5-HT after electrochemical HPLC and also the measurement of the extracellular ¹⁸F-MPPF radioactivity by counting the dialysis samples using a gamma counter in the same animal.

The ¹⁸F-MPPF radioactivity curves are well documented in PET scans in humans (Passchier *et al*. 2000a). After i.v. administration, ¹⁸F-MPPF accumulates preferentially in the medial temporal cortex, especially in the hippocampus area, with a ratio of approximately 3 with the cerebellum (Passchier *et al*. 2000b). Tomography of the rat brain with microPET revealed an average hippocampus/cerebellum ratio of 2, 30min after injection (Plenevaux *et al*. 2000a). Although SIC does not deliver images but defines only a detection volume drawn around the probe, our results demonstrate the validity and the feasibility of SIC for ¹⁸F-MPPF studies in rats. In our experiments, the hippocampus to cerebellum ratio increased linearly with time, reaching a value of 2 after 20 min and being reproducible between rats. No initial peak of radioactivity in the hippocampus or in the cerebellum was detected with the SIC probe, in contrast with that observed in PET scans. The initial peak of radioactivity is generally considered as the vascular bolus. Its absence in the SIC data suggest that SIC is less sensitive to the circulating radioactivity than a PET camera (Zimmer *et al*. 2002). The specificity of the ¹⁸F-MPPF binding on the 5-HT_{1A} receptors was confirmed by the total displacement of ¹⁸F-MPPF after injection of unlabelled WAY 100635, a specific 5-HT_{1A} antagonist. Simultaneously, after a ¹⁸F-MPPF injection, the measurement of the extracellular radioactivity after microdialysis collection revealed a higher radioactivity in the cerebellum than in the hippocampus. This radioactivity can be attributed to the ¹⁸F-MPPF itself since more than 90% of the radioactivity in the hippocampus and the cerebellum is due to the parent compound (Plenevaux *et al*. 2000b). This result could be interpreted as a lower free ¹⁸F-MPPF in extracellular space of the hippocampus as a fraction of ¹⁸F-MPPF is specifically bound on 5-HT_{1A} receptors.

In order to observe a ¹⁸F-MPPF displacement, we increased synaptic 5-HT levels by using fenfluramine. Microdialysis studies in rats have shown that fenfluramine increases hippocampal 5-HT concentrations to the micro-molar range (Thomas *et al*. 2000). Mechanistically, fenfluramine is known to cause a release in cytosolic 5-HT stores and inhibit its re-uptake (Rowland and Carlton 1986; Bonanno *et al*. 1994). In our studies, the microdialysis data and the β-kinetic measurement of ¹⁸F-MPPF were obtained simultaneously, thus providing the opportunity to examine the relationship between microdialysis and SIC measurements within each experiment. Our microdialysis results show the existence of a dose-effect relationship between challenge drug and extracellular 5-HT concentrations. The SIC measurements revealed that the magnitude of the ¹⁸F-MPPF-specific binding decreased gradually and proportionally with the magnitude of 5-HT concentration increase. A large increase in extracellular 5-HT release was therefore associated with a total displacement of the specific binding of ¹⁸F-MPPF, whereas the non-specific binding of ¹⁸F-MPPF in the cerebellum was unchanged.

Between 0 and 2 mg/kg fenfluramine injected, each per cent decrement in ¹⁸F-MPPF specific binding corresponded to a 1% increase of 5-HT concentration following fenfluramine injection. But this quantification must be interpreted with care as there is a 5-HT concentration gradient between the neuronal level and the extracellular fluid collected by microdialysis (Fisher *et al.* 1995; Di Chiara *et al.* 1996). Moreover, the extrasynaptic location of the 5-HT transporters (Zhou *et al.* 1998) allows the neurotransmitter to diffuse into the brain extracellular space, characterizing a paracrine neurotransmission (Bunin and Wightman 1999).

According to our results, our methodological approach allows the measurement of ¹⁸F-MPPF displacement at different levels in the rat hippocampus. Our interpretation is that ¹⁸F-MPPF is displaced from the 5-HT_{1A} receptors after a fenfluramine-increase of 5-HT release. The consequence is that ¹⁸F-MPPF is extracted from the neuronal level and is no longer detected by SIC. At this time, the displaced ¹⁸F-MPPF appears in the extracellular level, is collected by microdialysis and detected by gamma counting.

To our knowledge few data are available in the literature documenting the displacement of a PET radioligand by cerebral 5-HT. The available works concern mainly the widely used ¹¹C-WAY 100635 and no data with ¹⁸F-MPPF and endogenous 5-HT are published. Moreover, it must be noticed that a direct comparison with these works has to be done cautiously since different animal models or techniques were used. A first study had shown that pre-treatment by fenfluramine decreased the specific binding of the ¹¹C-WAY 100635 in rats and rhesus monkeys (Mathis *et al.* 1995). However, these results were limited as the dose of fenfluramine was high (8 mg/kg, i.v.) and the resulting tissular displacement low (< 20%). In another study in baboons, administration of the 5-HT releaser fenfluramine failed to decrease ¹¹C-WAY 100635-specific binding (Parsey *et al.* 1999). In a recent paper using a combination of microPET and microdialysis (Hume *et al.* 2001), it has been shown that the massive release of 5-HT after 10 mg/kg fenfluramine injection (15-fold increase, according to our results) induced a reduction of the ¹¹C-WAY 100635 of only 10-20% in the rat hippocampus. The author claimed that this minimal effect was explained by a low baseline occupancy of the 5-HT_{1A} receptors by 5-HT, so that only a large change in endogenous agonist concentration would affect radioligand binding. Although our radioactive measurement technique is different (SIC vs. microTEP), we cannot agree with this affirmation as our results showed that a small modification of the 5-HT extracellular concentration induced a significant displacement of ¹⁸F-MPPF, in the same animal model and with the same pharmacological paradigm. The initial proposition of Seeman *et al.* (1989), to the effect that low affinity radiotracers are more vulnerable to endogenous neurotransmitter competition, has gained wide acceptance. The dissociation of ¹⁸F-MPPF from the 5-HT_{1A} receptors is therefore clearly much more rapid than the dissociation of ¹¹C-WAY 100635, possibly explaining the lower affinity of MPPF (Zhuang *et al.* 1994). In this view, the high affinity of ¹¹C-WAY 100635 may restrict its use for measuring changes in endogenous 5-HT levels. However, according to Laruelle (2000), the simple binding competition might not be the only phenomenon regulating transmitter-radioligand interactions *in vivo*. The concept of receptor trafficking (internalization) might also be involved (Laruelle 2000; Laruelle and Huang 2001).

Our study constitutes the first encouraging observation for the serotonergic system, allowing a better understanding of the radiotracer binding vulnerability to changes in endogenous transmitter levels. A complementary study would be to investigate the effect of phasic or tonic effect of diminution of 5-HT content on the ¹⁸F-MPPF binding. This work is now in progress in our laboratory. Since it is known that some spontaneous behaviours (sleep, arousal, stress response, etc.) induce a 30-100% increase in 5-HT release in the rat hippocampus (for a review see Rueter *et al.* 1997), it would be of great interest to evaluate these paradigms during ¹⁸F-MPPF injection. Moreover, the possibility of the behavioural state of a patient during a PET scan having an indirect effect of the ¹⁸F-MPPF binding cannot be excluded.

In conclusion, we have established for the first time that ¹⁸F-MPPF-specific binding could reflect changes in cerebral 5-HT in the rat hippocampus. Our experiments presented showed that this displacement is well correlated with the enhanced extracellular 5-HT concentrations. This approach, combined with an appropriate pharmacological challenge paradigm, can be used to address a wide range of issues relevant to the regulation of neurotransmitter activity *in vivo*, mechanisms of neuropsychiatric disease or drug development in animal models. After modelization ¹⁸F-MPPF could constitute an original tool to quantify *in vivo* the cerebral 5-HT in limbic areas. Consequently, ¹⁸F-MPPF would be essential to realising the unique potential of the challenge techniques for measurement of 5-HT synaptic transmission in the living human brain.

References

- [1] Benveniste H. (1989) Brain microdialysis. *J. Neurochem.* 52, 1667-1679.
- [2] Bonanno G., Fassio A., Severi P., Ruelle A. and Raiteri M. (1994) Fenfluramine releases serotonin from human brain nerve endings by a dual mechanism. *J. Neurochem.* 63, 1163-1166.
- [3] Bunin M. A. and Wightman R. M. (1999) Paracrine neurotransmission in the CNS: involvement of 5-HT. *Trends Neurosci.* 22, 377-382.

- [4] Burnet P. W., Eastwood S. L., Lacey K. and Harrison P. J. (1995) The distribution of 5-HT_{1A} and 5-HT_{2A} receptor mRNA in human brain. *Brain Res.* 676, 157-168.
- [5] Di Chiara G., Tanda G. and Carboni E. (1996) Estimation of *in vivo* neurotransmitter release by brain microdialysis: the issue of validity. *Behav Pharmacol.* 7, 640-657.
- [6] Fisher R. E., Morris E. D., Alpert N. M. and Fischman A. J. (1995) *In vivo* imaging of neuromodulatory synaptic transmission using PET: a review of relevant neurophysiology. *Human Brain Mapp.* 3, 24-34.
- [7] Gardner J. P., Fomal C. A. and Jacobs B. L. (1997) Effects of sleep deprivation on serotonergic neuronal activity in the dorsal raphe nucleus of the freely moving cat. *Neuropsychopharmacology* 17, 72-81.
- [8] Ginovart N., Hassoun W., Le Bars D., Weissmann D. and Leveil V. (2000) *In vivo* characterization of p-[¹⁸F]MPPF, a fluoro analog of WAY-100635 for visualization of 5-HT_{1A} receptors. *Synapse* 35, 192-200.
- [9] Glennon R. A. (1990) Serotonin receptors: clinical implications. *Neurosci. Biobehav Rev.* 14, 35-7.
- [10] Hamon M., Lanfumey L., el Mestikawy S., Boni C., Miquel M. C., Bolanos F., Schechter L. and Gozlan H. (1990) The main features of central 5-HT₁ receptors. *Neuropsychopharmacology* 3, 349-360.
- [11] Hillver S. E., Bjork L., Li Y. L., Svensson B., Ross S., Anden N. E. and Hacksell U. (1990) (S)-5-Fluoro-8-hydroxy-2-(dipropylamino) tetralin: a putative 5-HT_{1A} receptor antagonist. *J. Med. Chem.* 33, 1541-1544.
- [12] Hume S., Hirani E., Opacka-Juffry J., Myers R., Townsend C., Pike V. and Grasby P. (2001) Effect of 5-HT on binding of [¹¹C]WAY 100635-5-HT_{1A} receptors in rat brain, assessed using *in vivo* microdialysis and PET after fenfluramine. *Synapse* 41, 150-159.
- [13] Ichise M., Meyer J. H. and Yonekura Y (2001) An introduction to PET and SPECT neuroreceptor quantification models. *J. Nucl. Med.* 42, 755-763.
- [14] Kia H. K., Miquel M. C., Brisorgueil M. J., Daval G., Riad M., El Mestikawy S., Hamon M. and Vergé D. (1996) Immunocytochemical localization of serotonin-1A receptors in the rat central nervous system. *J. Comp. Neurol.* 365, 289-305.
- [15] Kostowski W., Plaznik A. and Archer T. (1989) Possible implication of 5-HT_{1A} function for the etiology and treatment of depression. *New Trends Exp. Clin. Psychiatry* 5, 91-116.
- [16] Kung H. F., Stevenson D. A., Zhuang Z. P., Kung M. P., Frederick D. and Hurt S. D. (1996) New 5-HT_{1A} receptor antagonist: [³H]-P-MPPF. *Synapse* 4, 344-346.
- [17] Laruelle M. (2000) Imaging synaptic neurotransmission with *in vivo* binding competition techniques: a critical review. *J. Cereb. Blood Flow Metab.* 20, 423-451.
- [18] Laruelle M. and Huang Y (2001) Vulnerability of positron emission tomography radiotracers to endogenous competition. *Q. J. Nucl. Med.* 45, 124-138.
- [19] Le Bars D., Lemaire C., Ginovart N., Plenevaux A., Aerts J., Brihaye C., Hassoun W., Leveil V., Mekhsian P., Weissmann D., Pujol J. F., Luxen A. and Comar D. (1998) High yield radiosynthesis and preliminary *in vivo* evaluation of p-[¹⁸F]MPPF, a fluoro analog of WAY 100635. *Nucl. Med. Biol.* 25, 343-350.
- [20] Le Bars D., Bonmarchand G., Alvarez J. M., Lemaire C. and Mosdzianowski C. (2001) New automation of ¹⁸F-MPPF using a coincidence synthesizer. *J. Labelled Cpd. Radiopharm.* 44, S1045.
- [21] Mathis C., Simpson N., Price J., Mahmood K., Bowman K. and Mintun M. (1995) Evaluation of [¹¹C]WAY 100635: a potent serotonin 5-HT_{1A} receptor antagonist. *J. Nucl. Med.* 36, 162P (Abstract).
- [22] Meneses A. (1999) 5-HT system and cognition. *Neurosci. Biobehav. Rev.* 23, 111-1125.
- [23] Meneses A. and Hong E. (1997) Role of 5-HT_{1A} receptors in acquisition, consolidation and retrieval of learning. *CNS Drugs Rev.* 3, 68-82.
- [24] Pain F., Laniec P., Mastripolito R., Charon Y., Comar D., Leveil V., Pujol J. F. and Valentin L. (2000) SIC, an intracerebral radiosensitive probe for *in vivo* neuropharmacology investigations in small laboratory animals: theoretical considerations and physical characteristics. *IEEE Trans. Nucl. Sci.* 47, 25-32.
- [25] Palacios J. M., Waeber C., Hoyer D. and Mengod G. (1990) Distribution of serotonin receptors. *Ann. NY Acad. Sci.* 600, 36-52.
- [26] Parsey R. V., Hwang D. R., Simpson N., Guo N. N., Mawlawi O., Kegeles L. S., Bergert B., Montoya S., Popilskis J., Mann J. J. and Laruelle M. (1999) PET studies of competition between [³C]carbonyl-WAY 100635 and endogenous serotonin. *J. Nucl. Med.* 40, 29P (abstract).
- [27] Passchier J. and van Waarde A. (2001) Visualisation of serotonin-1A (5-HT_{1A}) receptors in the central nervous system. *Eur. J. Nucl. Med.* 28, 113-129.
- [28] Passchier J., van Waarde A., Pieterman R. M., Elsinga P. H., Pruijm J., Hendrikse N. H., Willemse A. T. and Vaalburg W. (2000a) *In vivo* delineation of serotonin (5-HT_{1A}) receptors in human brain with [¹⁸F]MPPF. *J. Nucl. Med.* 41, 1830-1835.
- [29] Passchier J., van Waarde A., Pieterman R. M., Elsinga P. H., Pruijm J., Hendrikse N. H., Willemse A. T. M. and Vaalburg W

- (2000b) Quantitative imaging of 5-HT_{1A} receptor binding in healthy volunteers with [¹⁸F]-MPPF. *Nucl. Med. Biol.* 24, 473-76.
- [30] Paxinos G. and Watson C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, New York.
- [31] Pazos A., Probst A. and Palacios J. M. (1987) Serotonin receptors in the human brain. III: Autoradiographic mapping of serotonin-1 receptors. *Neuroscience* 21, 97-122.
- [32] Peroutka S. J. (1995) Serotonin receptor subtypes. Their evolution and clinical relevance. *CNS Drugs* 4, 18-28.
- [33] Plenevaux A., Lemaire C., Aerts J., Lacan G., Rubins D., Melega W. P., Brihaye C., Degueldre C., Fuchs S., Salmon E., Maquet P., Laureys S., Damhaut P., Weissmann D., Le Bars D., Pujol J. F. and Luxen A. (2000a) [¹⁸F]p-MPPF: a radiolabelled antagonist for the study of 5-HT_{1A} receptors with PET. *Nucl. Med. Biol.* 27, 467-471.
- [34] Plenevaux A., Weissmann D., Aerts J., Lemaire C., Brihaye C., Degueldre C., Le Bars D., Comar D., Pujol J. F. and Luxen A. (2000b) Tissue distribution, autoradiography and metabolism of 4-(2'-methoxyphenyl)-l-[N-(2"-pyridinyl)-p-[¹⁸F]fluorobenzamido]ethyl]piperazine (p-[¹⁸F]MPPF), a new serotonin 5-HT_{1A} antagonist for positron emission tomography: an *in vivo* study in rats. *J. Neurochem.* 75, 803-811.
- [35] Rowland N. E. and Carlton J. (1986) Neurobiology of an anorectic drug: fenfluramine. *Prog. Neurobiol.* 27, 13-62.
- [36] Rueter L. E., Fornal C. A. and Jacobs B. L. (1997) A critical review of 5-HT brain microdialysis and behavior. *Rev. Neurosci.* 8, 117-137.
- [37] Saudou F. and Hen R. (1994) 5-Hydroxytryptamine receptor subtypes in vertebrates and invertebrates. *Neurochem. Int.* 25, 503-532.
- [38] Saxena P. R. (1995) Serotonin receptors: subtypes, functional response and therapeutic relevance. *Pharmacol. Ther.* 66, 339-368.
- [39] Seeman P., Guan H. C. and Niznik H. B. (1989) Endogenous dopamine lowers the dopamine D2 receptor density as measured by [³H]raclopride: implications for positron emission tomography of the human brain. *Synapse* 3, 96-97.
- [40] Seifritz E., Stahl S. M. and Gillin J. C. (1997) Human sleep EEG following the 5-HT_{1A} antagonist pindolol: possible disinhibition of the raphe neuron activity. *Brain Res.* 759, 84-91.
- [41] Shiue C. Y., Shiue G. G., Mozley P. D., Kung M. P., Zhuang Z. P., Kim H. J. and Kung H. F. (1997) p-[¹⁸F]MPPF: a potential radioligand for PET studies of 5-HT_{1A} receptors in humans. *Synapse* 25, 147-154.
- [42] Thielen R., Fangon N. B. and Frazer A. (1996) 4-(2'-methoxyphenyl)-l-[N-(2"-pyridinyl)-p-iodobenzamido]ethyl]piperazine and 4-(2'-methoxyphenyl)-l-[2"-pyridinyl]-p-fluorobenzamido]ethyl] piperazine, two new antagonist at pre- and postsynaptic serotonin-1A receptors. *J. Pharmacol. Exp. Ther.* 277, 661-670.
- [43] Thomas H., Fink H., Sohr T. R. and Voits M. (2000) Lesion of the median raphe nucleus: a combined behavioral and microdialysis study in rats. *Pharmacol. Biochem. Behav.* 65, 15-21.
- [44] Van Wijngaarden I., Tulp M. T and Soudijn W (1990) The concept of selectivity in 5-HT receptor research. *Eur. J. Pharmacol.* 188, 301-312.
- [45] Zhou F. C., Tao-Cheng J. H., Segu L., Patel T and Wang Y (1998) Serotonin transporters are located on the axons beyond the synaptic junctions: anatomical and functional evidence. *Brain Res.* 805, 241-254.
- [46] Zhuang Z. P., Kung M. P. and Kung H. F. (1994) Synthesis and evaluation of 4-(2'-methoxyphenyl)-l-[2"-pyridinyl]-p-iodobenzamido]ethyl]piperazine (p-MPPI): a new iodinated 5-HT_{1A} ligand. *J. Med. Chem.* 37, 1406-1407.
- [47] Zimmer L., Hassoun W., Pain F., Bonnefoi F., Lanièce P., Mastripolito R., Pinot L., Pujol J. F. and Leviel V (2002) SIC, an intracerebral B⁻-range-sensitive probe for radiopharmacology investigations in small laboratory animals: binding studies with [¹¹C]raclopride. *J. Nucl. Med.* 43, in press.