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Effect of Increased Serotonin Levels on [^{18}F]MPPF Binding in Rat Brain: Fenfluramine *vs* the Combination of Citalopram and Ketanserin

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[^{18}F]MPPF is a selective serotonin-1A (5-HT$_{1A}$) receptor antagonist and may be used to measure changes in the functional levels of serotonin (5-HT). The technique is based on the assumption that the injected radiolabeled ligand competes for the same receptor as the endogenous transmitter. Results from studies using serotonergic ligands are not always consistent. The aim of the present study was to investigate if [^{18}F]MPPF binding is decreased after an increase in 5-HT levels. [^{18}F]MPPF binding was assessed in conscious rats using *ex vivo* autoradiography. We studied the effect of the 5-HT-releasing agent and reuptake inhibitor fenfluramine (10 mg/kg i.p.) and of a combination of the selective serotonin reuptake inhibitor (SSRI) citalopram (10 μmol/kg, s.c.) with the 5-HT$_{2C}$ antagonist ketanserin (100 nmol/kg, s.c). The effect of both treatments on extracellular 5-HT levels was determined using microdialysis. Fenfluramine treatment resulted in a 30-fold increase in extracellular 5-HT levels in the ventral hippocampus and induced a significant reduction of [^{18}F]MPPF binding in the frontal cortex, hypothalamus, amygdala, and hippocampus. The microdialysis results showed a 10-fold 5-HT increase in the ventral hippocampus after combined administration of ketanserin and citalopram. The combination, however, did not affect [^{18}F]MPPF binding. Our data show that [^{18}F]MPPF binding in conscious rats is only reduced after substantial and therefore nonphysiological increases in 5-HT levels. These results may imply that the majority of 5-HT$_{1A}$ receptors is in the low-affinity state, *in vivo*.
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INTRODUCTION

The serotonergic system has been implicated in the pathophysiology and treatment of a variety of psychiatric disorders such as depression, anxiety, and schizophrenia (Blier and de Montigny, 1998; den Boer, 2000; Kapur and Remington, 2001; Seeman, 2002). Several studies reported the possible involvement of the 5-HT$_{1A}$ receptor in these disorders (Bantick *et al*, 2001; Groenink *et al*, 2003; Hjorth *et al*, 2000). Differences in receptor densities can be quantified using radiolabeled ligands. The selective 5-HT$_{1A}$ receptor antagonists [^{18}F]MPPF (4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-[^{18}F]fluorobenzamido]ethylpiperazine) and [^{11}C]WAY-100635 ([^{11}C][*O*-methyl-3*H*]-*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride) appear to be useful radioligands for imaging of the 5-HT$_{1A}$ receptor in human subjects (Andree *et al*, 2000; Passchier *et al*, 2000; Sargent *et al*, 2000).

Previous studies have shown that radiolabeled ligands could also be used to measure changes in the functional level of neurotransmitters in the brain. Abnormalities in 5-HT transmission have been widely studied using neuroendocrine challenge studies (Power and Cowen, 1992). This method, however, only reflects functioning of the hypothalamo-hypophysial serotonergic system, but does not necessarily assess 5-HT transmission in other brain regions. The combined use of radiolabeled ligands with a serotonergic challenge may provide information on 5-HT release in specific regions of the brain. The approach is based on the assumption that an injected radiolabeled ligand competes for the same receptor as the endogenous transmitter. So, increases in neurotransmitter release result in a decreased binding of the radioligand and decreased neurotransmitter

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release induces an increase in ligand binding. The changes in ligand binding are used as a measure of the change in neurotransmitter levels. This method has successfully been used for the dopaminergic system (Breier *et al*, 1997; Laruelle, 2000). Results from studies using serotonergic ligands, however, do not always agree.

At present, a few studies have investigated if the binding of serotonergic ligands is sensitive to changes in 5-HT levels. These studies have primarily used [^{11}C]WAY-100635 and [^{18}F]MPPF and have been performed in rats and human subjects. Hume *et al* (2001) reported the effect of the 5-HT-releasing agent and reuptake inhibitor fenfluramine (10 mg/kg i.p.) on [^{11}C]WAY-100635 binding. They investigated the effect by means of positron emission tomography (PET) in anesthetized rats and the *ex vivo* distribution in dissected brain tissues from nonanesthetized rats. The PET results showed a 20% decrease in [^{11}C]WAY-100635 binding potential in the hippocampus but not in the prefrontal cortex or raphe nucleus. The post-mortem dissection studies did not show a statistically significant effect of fenfluramine on [^{11}C]WAY-100635 uptake in the majority of tissues sampled, probably due to the relative long time interval between fenfluramine administration and measurement of radioactivity content. Using the same radioligand and comparable methods, Maeda *et al* (2001) did not find an effect of fenfluramine (10 mg/kg i.p.) in the hippocampus of anesthetized rats. Zimmer *et al* (2002a) investigated the effect of different doses of fenfluramine on [^{18}F]MPPF binding in anesthetized rats, using a β^+ radiosensitive probe. The authors reported a dose-related decrease of [^{18}F]MPPF binding in the hippocampus. In human subjects, [^{11}C]WAY-100635 binding in the prefrontal cortex and medial temporal cortex was not consistently affected after manipulation of 5-HT levels by means of either tryptophan depletion or tryptophan infusion (Rabiner *et al*, 2002). We have studied the effect of changes in 5-HT release on [^{18}F]MPPF binding in human subjects and did not find a significant difference in [^{18}F]MPPF binding between a tryptophan depletion and tryptophan infusion condition (Udo de Haes *et al*, 2002).

The lack of effect in the studies that manipulated tryptophan levels may have been caused by the fact that intrasynaptic 5-HT levels are not sufficiently changed to produce a measurable effect on ligand binding (Rabiner *et al*, 2002; Udo de Haes *et al*, 2002). The differences in the studies using fenfluramine may be related to differences in timing of the pharmacological treatment. Another important factor could be the use of anesthesia. Previous studies have shown that ligand binding may be affected by the use of different anesthetics. The mechanism of these effects is not completely understood but may be related to changes in cerebral blood flow or receptor affinity (Ginovart *et al*, 2002; Harada *et al*, 2004; Hassoun *et al*, 2003; Seeman and Kapur, 2003). The size of 5-HT increase may also differ between conscious and anesthetized rats (Mokler *et al*, 1998).

In the present study we investigated the effect of fenfluramine (10 mg/kg, i.p.) and of a combination of the SSRI citalopram with the 5-HT$_{2C}$ antagonist ketanserin. Microdialysis studies in rat have shown that both treatments induce marked increases in extracellular 5-HT concentrations. The effect of the combined treatment of citalopram with ketanserin may be caused by a combination of 5-HT reuptake inhibition and modulation of global or local feedback mechanism(s), resulting in increased 5-HT release (Cremers *et al*, 2004). Since, as mentioned before, anesthetics may have confounding effects on ligand binding, we used conscious rats. The effects on [^{18}F]MPPF binding were investigated using *ex vivo* autoradiography. Concurrently, the effect of both challenges on extracellular 5-HT concentration was studied using similar dosages in microdialysis experiments.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–350 g (Harlan, Zeist, The Netherlands) were used. After surgery (see below), rats were housed individually and kept on a 12-h light/dark schedule with food and water *ad libitum*. Microdialysis and *ex vivo* autoradiography experiments were carried out in separate animal groups. During microdialysis sampling and radioligand injection, rats were in conscious condition and able to move freely in their cages. All experiments were carried out during the light period. The study was approved by the Animal Care Committee of the University of Groningen.

Synthesis of [^{18}F]MPPF

[^{18}F]MPPF was prepared by nucleophilic [^{18}F] fluorination of the appropriate nitro precursor (see Shiue *et al* (1997) for a comparable method). It was formulated into a 5% NaCl solution. Levels of nitro precursor were $<<1$ mg/l. The radiochemical purity was greater than 95% and the specific activity >10 TBq/mmol at the time of injection.

Ex Vivo Autoradiography

Jugular veins were cannulated 24–48 h prior to radioligand injection (for a detailed description of the cannulation method, see Steffens (1969), for details on anesthesia during cannulation, see microdialysis experiments). At 2 h before radioligand injection, the animals were deprived of food. Rats were injected with [^{18}F]MPPF via the jugular vein cannula, in conscious condition. The mean ($\pm$SD) injected activity and injected mass were 14.4 ($\pm$5.8) MBq and 0.23 ($\pm$0.07) nmol, respectively, and did not significantly differ between groups. At 30 min prior to radioligand injection, the animals were treated either with saline ($n=7$), fenfluramine (10 mg/kg, i.p.) ($n=7$), or a combination of citalopram (10 μmol/kg, s.c.) with ketanserin (100 nmol/kg, s.c.) ($n=5$). The animals were killed by rapid guillotine decapitation (no anesthesia) 30 min after [^{18}F]MPPF administration. This time point is based on earlier studies which showed that 30 min after injection, MPPF binding reaches a state of transient equilibrium (Plenevaux *et al*, 2000b; Shiue *et al*, 1997). The brains were removed from the skull, frozen in isopentane (−80°C), cut into 80 μm coronal slices in a cryostat at −10°C, thaw mounted onto glass slides, exposed to a phosphor storage screen (Packard) for at least 10 half-life times (18–20 h) and scanned using the Cyclone storage phosphor system. Regions of interest (ROIs) were drawn

around the frontal cortex, cingulate cortex, septal nuclei, caudate-putamen (striatum), thalamus, hypothalamus, dentate gyrus, interpeduncular nucleus, amygdala, hippocampus, dorsal raphe, and median raphe, using the Paxinos and Watson brain atlas (Paxinos and Watson, 1998). For each region, data from the four sections with the highest activity were averaged, except for the amygdala, interpeduncular nucleus, and raphe where an average of two sections was used. For quantification, the digital light units (DLU)/mm^2 values were measured for the different ROIs. Individual calibration standards with known activity were exposed to the screen simultaneously with the brain slices to convert the DLU/mm^2 values to Bq/mm^2. The activity of the different regions was converted to % injected dose per gram tissue (%ID) by dividing the regional activity by the injected activity and thickness of the slice. Specific binding was defined by the activity ratio of the region of interest to the cerebellum, a region virtually devoid of 5-HT$_{1A}$ receptors (Hall *et al*, 1997).

Microdialysis Experiments

Preceding surgery, rats were anesthetized by means of isoflurane 2%, 600 ml/min O_2, and 400 ml/min N_2O. Microdialysis probes were inserted in the ventral hippocampus (L +4.8 mm, IA: +3.7 mm, V: −8.0 mm) and dorsal raphe nucleus (L −1.4 mm, IA: +1.2 mm, V: −7.0 mm angle 10°). Sample collection was performed 24–48 h after surgery, in conscious condition. The animals were treated either with fenfluramine (10 mg/kg, i.p.) ($n=4$ in the ventral hippocampus) or a combination of citalopram (10 μmol/kg, s.c.) with ketanserin (100 nmol/kg, s.c.) ($n=4$ in the ventral hippocampus and raphe nucleus). The probes were perfused with artificial cerebrospinal fluid containing (in mM): NaCl 147, KCl 3.0, $CaCl_2$ 1.2, and $MgCl_2$ 1.2, at a flow-rate of 1.5 l/m). Microdialysis samples (15-minute) were collected and 5-HT levels were measured by HPLC with electrochemical detection. Post mortem, the position of the probe was verified by the track of the probe through the brain. Data are expressed as percent baseline. For a more detailed description of the microdialysis experiments and serotonin analysis, see Cremers *et al* (2004).

Behavioral Observation

The animals were continuously observed after injection of the pharmacological treatments, and effects on body movements or posture were scored as present if occurring during the observation period.

Statistics

The effects of the different treatments on [^{18}F]MPPF binding were analyzed by an independent samples *t*-test. The data are presented as mean ratio to cerebellum (±SD) and % change compared to control rats. Bonferroni corrections were used in order to correct for multiple comparisons.

Drugs

Citalopram hydrobromide and racemic fenfluramine hydrochloride were synthesized at and obtained from Lundbeck A/S (Copenhagen, Denmark). Ketanserin was obtained from RBI (Natick, USA). All drugs were dissolved in saline.

RESULTS

Microdialysis

Fenfluramine (10 mg/kg, i.p.) administration induced a 25-fold increase in the hippocampus at 45 min postinjection. 5-HT levels were maximal at 60 min after administration. At that time, a 30-fold increase in 5-HT levels was observed. After combined administration of citalopram (10 μmol/kg, s.c.) and ketanserin (100 nmol/kg, s.c.), a 10-fold increase in 5-HT was observed in the ventral hippocampus and a five-fold increase in the raphe nucleus. Peak levels were achieved 45 min after administration (Figure 1).

Ex Vivo Autoradiography

[^{18}F]MPPF distribution. After administration of [^{18}F]MPPF, the distribution of radioactivity in the control group was in agreement with previous results and with known 5-HT$_{1A}$ receptor localization, with the highest

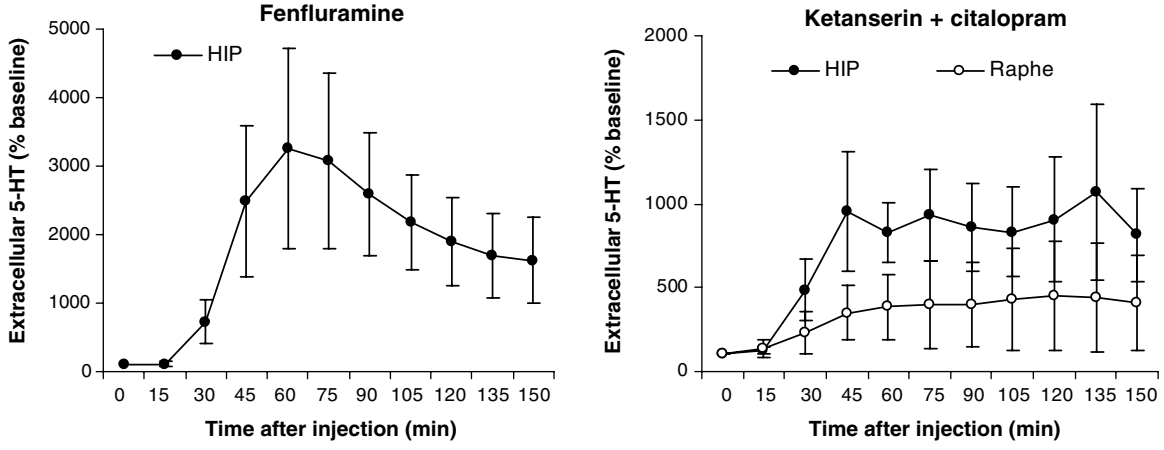

Figure 1 Mean (±SD) extracellular 5-HT levels after fenfluramine (10 mg/kg, i.p.) ($n=4$) (ventral hippocampus) and combined citalopram (10 μmol/kg, s.c.) with ketanserin (100 nmol/kg, s.c.) ($n=4$) (ventral hippocampus and dorsal raphe nucleus) administration to conscious rats. HIP: hippocampus.

uptake in the raphe nuclei, septum, and hippocampus and low uptake in the cerebellum (Ginovart *et al*, 2000; Passchier *et al*, 2000; Plenevaux *et al*, 2000a; Shiue *et al*, 1997). Region over cerebellum ratios ranged from approximately 1 in the striatum to around 10 in the dorsal raphe nucleus.

Effect of fenfluramine pretreatment. Administration of fenfluramine did not significantly affect cerebellar [^{18}F]MPPF binding, compared to control rats. In fenfluramine-treated rats, mean ($\pm$ SD) radioactivity in the cerebellum was 0.028 ($\pm$0.017) %ID, compared to 0.030 ($\pm$0.010) %ID in control rats. Figure 2 depicts an example of a phosphor screen image at the level of the hippocampus and cerebellum of a control and fenfluramine-treated rat, showing that [^{18}F]MPPF binding is reduced in the fenfluramine-treated rat compared to the control rat. In Table 1 and Figure 3, the mean [^{18}F]MPPF-binding values (ratio to cerebellum) of control and fenfluramine-treated rats are shown. Fenfluramine treatment resulted in a significant reduction in [^{18}F]MPPF binding in the frontal cortex, thalamus, hypothalamus, amygdala, dentate gyrus, hippocampus, and raphe nuclei. After Bonferroni correction, the reduction was significant in the frontal cortex, hypothalamus, amygdala, and hippocampus.

Effect of ketanserin–citalopram pretreatment. Combined administration of ketanserin with citalopram also did not have a significant effect on cerebellar [^{18}F]MPPF binding, compared to control rats. In the ketanserin with citalopram-treated rats, mean ($\pm$SD) radioactivity in the cerebellum was 0.029 ($\pm$0.006) %ID, compared to 0.030 ($\pm$0.010) %ID in control rats. Table 1 and Figure 3 show mean [^{18}F]MPPF-binding values (ratio to cerebellum) of control and ketanserin with citalopram-treated rats. In contrast to the effects of fenfluramine, pretreatment with ketanserin and citalopram did not significantly affect [^{18}F]MPPF binding, except in the dorsal raphe nucleus. After Bonferroni correction, no significant changes were found.

Effects on Behavior

Fenfluramine administration resulted in hind leg abduction, straub tail, penile licking, and increased respiration. After administration of ketanserin and citalopram, penile erections were observed and an increase in grooming and licking behavior. Effects were seen at the time of

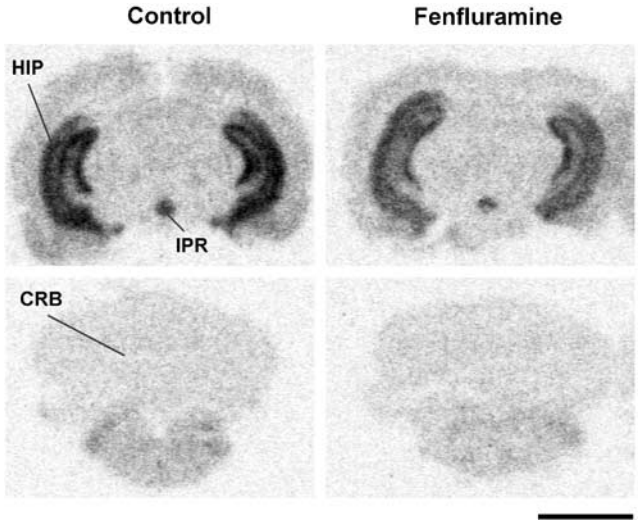

Figure 2 *Ex vivo* phosphor screen images, 30 min after i.v. injection of [^{18}F]MPPF to conscious rats. Example of coronal sections at the level of the hippocampus and cerebellum in saline or fenfluramine (10 mg/kg, i.p.)-pretreated animals. HIP: hippocampus, IPR: interpeduncular nucleus, CRB: cerebellum. Bar: 5 mm.

Table 1 Mean ($\pm$SD) Regional *Ex vivo* [^{18}F]MPPF Binding (Ratio to Cerebellum), 30 min after i.v. Injection of the Ligand to Conscious Rats

	[^{18}F]MPPF binding (ratio to cerebellum)		
Region	**Control ($n=7$) Mean$\pm$SD**	**Fenfluramine ($n=7$) Mean$\pm$SD (% change)**	**Ketanserin+citalopram ($n=5$) Mean$\pm$SD (% change)**
Frontal cortex	4.0$\pm$0.3	3.0$\pm$0.3 (−23%)**	4.1$\pm$0.4 (+3%)
Cingulate cortex	3.4$\pm$0.5	2.9$\pm$0.4 (−12%)	3.5$\pm$0.4 (+4%)
Septum	9.7$\pm$1.7	8.4$\pm$0.6 (−13%)	10.1$\pm$1.5 (+4%)
Striatum	1.1$\pm$0.1	1.0$\pm$0.1 (−5%)	1.0$\pm$0.1 (−3%)
Thalamus	1.3$\pm$0.1	1.1$\pm$0.1 (−14%)	1.3$\pm$0.1 (+5%)
Hypothalamus	2.5$\pm$0.4	1.7$\pm$0.4 (−33%)*	2.5$\pm$0.6 (−1%)
Dentate gyrus	8.3$\pm$1.3	6.7$\pm$0.4 (−19%)	8.3$\pm$1.0 (−0%)
Amygdala	4.8$\pm$0.3	3.6$\pm$0.4 (−24%)**	4.6$\pm$0.9 (−3%)
Hippocampus	8.8$\pm$1.0	7.1$\pm$0.7 (−20%)*	8.8$\pm$1.2 (−0%)
Interpeduncular nucleus	6.2$\pm$1.2	5.8$\pm$0.4 (−7%)	6.4$\pm$1.1 (+3%)
Dorsal raphe	10.3$\pm$1.3	7.8$\pm$1.8 (−24%)	8.5$\pm$0.7 (−17%)
Median raphe	3.9$\pm$0.6	3.0$\pm$0.5 (−23)	3.6$\pm$0.6 (−7%)

% change refers to drug- *versus* saline-treated rats. *Indicates significant difference compared to control rats ($p<0.05$, two-tailed *t*-test with Bonferroni correction). **Indicates significant difference compared to control rats ($p<0.01$, two-tailed *t*-test with Bonferroni correction).

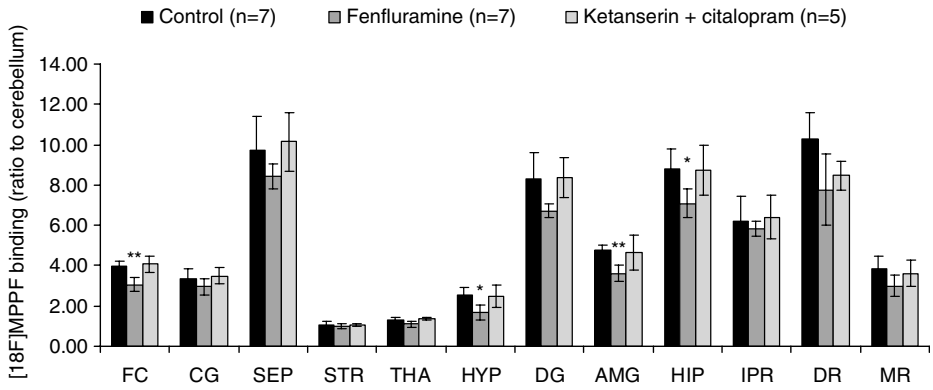

Figure 3 Mean (±SD) regional *ex vivo* [^{18}F]MPPF binding (ratio to cerebellum), 30 min after i.v. injection of the ligand to conscious rats. [^{18}F]MPPF was administered 30 min after saline, fenfluramine (10 mg/kg, i.p.), or combined citalopram (10 μmol/kg, s.c.) with ketanserin (100 nmol/kg, s.c.) treatment. *Indicates significant difference compared to control rats ($p<0.05$, two-tailed *t*-test with Bonferroni correction). **Indicates significant difference compared to control rats ($p<0.01$, two-tailed *t*-test with Bonferroni correction). FC: frontal cortex, CG: cingulate cortex, SEP: septum, STR: striatum, THA: thalamus, HYP: hypothalamus, DG: dentate gyrus, AMG: amygdala, HIP: hippocampus, IPR: interpeduncular nucleus, DR: dorsal raphe, MR: median raphe.

[^{18}F]MPPF injection and were still present at the moment of decapitation.

DISCUSSION

The aim of this study was to investigate if [^{18}F]MPPF binding to the 5-HT$_{1A}$ receptor is reduced after large increases in extracellular 5-HT. We have shown that the administration of fenfluramine resulted in a significant reduction of [^{18}F]MPPF binding in the frontal cortex, hypothalamus, amygdala, and hippocampus of conscious rats. [^{18}F]MPPF binding was not changed after combined administration of ketanserin and citalopram.

Previous *ex vivo* dissection studies in nonanesthetized and anesthetized animals (Hume *et al*, 2001; Maeda *et al*, 2001, respectively) did not show a significant effect of fenfluramine on the distribution of [^{11}C]WAY-100635. However, in both studies, a nonsignificant reduction in radioactivity content was seen in several brain areas, comparable to the regions in our study. The differences in effect size between our study and their studies may be explained by differences in method or timing of the pharmacological challenge. In our study, the animals were killed at the time of the peak in 5-HT concentration, whereas in the studies of Maeda *et al* (2001) and Hume *et al* (2001), the animals were killed before or after the peak in 5-HT response, respectively. Using PET, Hume *et al* (2001) reported a 20% reduction in [^{11}C]WAY-100635 binding potential in the hippocampus after 10 mg/kg fenfluramine, a reduction comparable to that seen in our study. Using the same ligand as used in our study, the group of Zimmer studied the effect of changes in 5-HT levels by administration of different doses of fenfluramine (Zimmer *et al*, 2002a, b). [^{18}F]MPPF binding was studied in anesthetized rats using a β^+ radiosensitive probe. The effect of fenfluramine in their study was much larger than the effect as reported in our study. In the studies of Zimmer *et al*, a complete displacement of [^{18}F]MPPF was seen after an injection of 10 mg/kg fenfluramine. And even after lower doses of fenfluramine, reductions of 25–60% were seen. Compared to [^{11}C]WAY-100635, [^{18}F]MPPF has a much lower affinity for the 5-HT$_{1A}$ receptor (Ki of 0.8 and 3.3 nM, respectively) (Zhuang *et al*, 1994). According to Zimmer *et al* (2002a), [^{18}F]MPPF may therefore be more suitable for detection of changes in endogenous 5-HT. Other investigators, however, state that changes in specific binding are not dependent on the affinity of the ligand, if the experiment is performed at tracer doses (Abi-Dargham *et al*, 1999; Laruelle 2000). Therefore, it is not certain whether the large displacement of [^{18}F]MPPF in the studies of Zimmer *et al* (2002a, b) could be attributed to its lower affinity. Although the exact reason for the discrepancies between our study and the group of Zimmer is not clear, it may be due to methodological factors. The group of Zimmer investigated the effects on [^{18}F]MPPF binding using a β^+ radiosensitive probe, which is an invasive instrument and also sensitive to methodological errors (Ginovart *et al*, 2004). Previous autoradiography and PET studies using dopaminergic ligands have reported reductions in radioligand binding in the same order as in our study (for a review, see Laruelle, 2000).

Before attributing the effects of fenfluramine on [^{18}F]MPPF binding to an increase in intrasynaptic 5-HT release, we should also consider other possibilities to explain our results. The effect of fenfluramine in our study could have been caused by direct binding of this drug to the 5-HT$_{1A}$ receptor; however, this is not very likely since the affinity of fenfluramine for the 5-HT$_{1A}$ receptor is very low (μM range) (Mennini *et al*, 1991). Furthermore, the pharmacological treatments may have affected nonspecific binding. However, this would have caused changes in cerebellar activity, which is not significantly affected in our study. Increases in 5-HT levels may also have an effect on regional cerebral blood flow (Cohen *et al*, 1996) which could have induced a decrease in [^{18}F]MPPF binding. However, if one assumes the reduction in [^{18}F]MPPF binding to be a general effect of increased 5-HT levels on blood flow, one would have expected an effect of the combination of ketanserin and citalopram as well, since citalopram by itself is already able to significantly change rCBF (McBean *et al*, 1999). Therefore, the effect of fenfluramine in our study

may indeed be explained by a reduced 5-HT_{1A} receptor availability, due to an increase in intrasynaptic 5-HT levels.

The binding of [^{18}F]MPPF was not affected by combined administration of citalopram and ketanserin. After administration of fenfluramine, a 30-fold increase in extracellular 5-HT levels was found, whereas administration of the combination only resulted in a 10-fold increase in 5-HT levels. Previous studies have shown that extracellular levels do not always reflect intrasynaptic processes (Tsukada *et al*, 2000a, b). The 5-HT_{1A} receptors are located both within the synapse and extrasynaptically (Azmitia *et al*, 1996; Riad *et al*, 2000). In a previous study using [^{18}F]MPPF, we concluded that changes in the binding of this ligand mainly reflect changes in intrasynaptic 5-HT levels, since, at least in postsynaptic areas, the proportion of extrasynaptic receptors is assumed to be low (Udo de Haes *et al*, 2002). The two treatments used in our study may have differently affected 5-HT levels at the intrasynaptic 5-HT_{1A} receptor due to their different regulation of 5-HT release and reuptake.

If we assume, however, that the extracellular 5-HT levels are a reflection of the intrasynaptic levels, our results may also be explained by the difference in the magnitude of the 5-HT increase. The effect of an increase in 5-HT on ligand binding can be calculated using the standard competition formula that relates the bound radiotracer (B) to the receptor density (B_{max}), the radioligand K_D, and free radioligand (L) in the presence of a competitor such as 5-HT, present at concentration F_{5-HT} and with an affinity K_i: $B = (B_{max}L)/(K_D\ (1 + F_{5-HT}/K_i) + L)$ (Abi-Dargham *et al*, 1999). When the ligand is administered at a tracer dose, L is negligible compared to K_D. Neglecting L in the denominator, and defining the binding potential (BP) before and after the serotonergic challenge as BP_1 and BP_2, F_{5-HT1} and F_{5-HT2} as the free 5-HT concentrations before and after the challenge, respectively, the relative reduction of BP induced by the challenge can be calculated as follows: $BP_2/BP_1 = (1 + F_{5-HT1}/K_i)/(1 + F_{5-HT2}/K_i)$. The relative change in BP induced by the change in F_{5-HT}, will be independent of K_D, as this factor cancels out. The 5-HT_{1A} receptor can exist in a high- and low-affinity state (Khawaja, 1995; Watson *et al*, 2000). In our calculations, we used 5-HT K_i values of 5 and 250 nM for the high- and low-affinity state, respectively (Watson *et al*, 2000) and a baseline extracellular 5-HT concentration of 0.9 nM (Cremers *et al*, 2004). Assuming all 5-HT_{1A} receptors to be in the high-affinity state, the calculated reduction in BP would be 82% after the 30-fold increase in 5-HT induced by fenfluramine and 58% after the 10-fold increase induced by the administration of ketanserin with citalopram. If all receptors would have been in the low-affinity state, the reduction in [^{18}F]MPPF binding would have been 9% after fenfluramine administration and 3% after the combination of ketanserin with citalopram. These data indicate that we would have seen an effect after the combination if a large proportion of the receptors would have been in the high-affinity state. Therefore, based on these calculations, we may conclude that the majority of 5-HT_{1A} receptors is in the low-affinity state.

Without Bonferroni correction, the combination of ketanserin and citalopram had a significant effect on [^{18}F]MPPF binding in the raphe nucleus, despite relatively small increases in 5-HT. This nucleus is very small and therefore the reduction may be a methodological artefact. However, other explanations are possible as well. In contrast to postsynaptic areas, in the raphe nucleus, a large proportion of receptors is located extrasynaptically (Kia *et al*, 1996). We expect the major part of extrasynaptic 5-HT_{1A} receptors to be in the agonist high-affinity state (Udo de Haes *et al*, 2002), and therefore 5-HT may have affected [^{18}F]MPPF binding in this nucleus. The reduction in the raphe may also be due to internalization of the 5-HT_{1A} receptor after agonist stimulation. Riad *et al* (2004) has shown that presynaptic receptors are internalized after agonist stimulation, whereas postsynaptic receptors are not.

To summarize, we have shown that the administration of fenfluramine resulted in a significant reduction of [^{18}F]MPPF binding in several brain areas of conscious rats. The combination of ketanserin and citalopram did not affect [^{18}F]MPPF binding, despite a considerable increase in extracellular 5-HT levels. Although possible effects of blood flow cannot be excluded, our data indicate that [^{18}F]MPPF binding is only reduced after large and therefore non-physiological increases in 5-HT levels. These results may imply that the majority of 5-HT_{1A} receptors is in the low-affinity state, *in vivo*.

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