In vivo imaging of dopamine and serotonin release
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CHAPTER 3

EFFECT OF INCREASED SEROTONIN LEVELS ON $[^{18}\text{F}]\text{-MPPF}$ BINDING IN RAT BRAIN:
FENFLURAMINE VS. THE COMBINATION OF CITALOPRAM AND KETANSERIN

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Abstract

[\textsuperscript{18}F]-MPPF is a selective serotonin-1A (5-HT\textsubscript{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 [\textsuperscript{18}F]-MPPF binding is decreased after an increase in 5-HT levels. [\textsuperscript{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 \textmu mol/kg, s.c.) with the 5-HT\textsubscript{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 [\textsuperscript{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 [\textsuperscript{18}F]-MPPF binding. Our data show that [\textsuperscript{18}F]-MPPF binding in conscious rats is only reduced after substantial and therefore non-physiological increases in 5-HT levels. These results may imply that the majority of 5-HT\textsubscript{1A} receptors is in the low affinity state, in vivo.
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 $[^{18}F]${\textsuperscript{}}MPPF (4-(2'-methoxyphenyl)-1-[2'-(N-2"-pyridinyl)-p-$[^{18}F]$fluorobenzamido]ethylpiperazine) and $[^{11}C]${\textsuperscript{}}WAY-100635 ($[^{11}C]${\textsuperscript{}}O-methyl-3H]-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 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 non-anesthetized 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 \(\beta^+\) 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
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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\textsubscript{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). Because, as mentioned before, anesthetics may have confounding effects on ligand binding, we used conscious rats. The effects on \textsuperscript{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 gram (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 done 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 done 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 to 48 hours prior to radioligand injection (for a detailed description of the cannulation method, see Steffens (1969), for details on anesthesia during cannulation, see microdialysis experiments). Two hours 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 ($±$ SD) injected activity and injected mass were 14.4 ($± 5.8$) MBq and 0.23 ($± 0.07$) nmol respectively and did not significantly differ between groups. 30 minutes 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 sacrificed by rapid guillotine decapitation (no anesthesia) 30 minutes after $[^{18}F]$-MPPF administration. This time point is based on earlier studies which showed that 30 minutes after injection, MPPF binding reaches a state of transient equilibrium (Plenevaux et al. 2000a; 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 hours) and scanned using the Cyclone storage phosphor system. Regions of interest (ROI’s) 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 4 sections with the highest activity were averaged, except for the amygdala, interpeduncular nucleus and raphe where an average of 2 sections was used. For quantification, the digital light units (DLU)/mm² values were measured for the different ROI’s. Individual calibration
standards with known activity were exposed to the screen simultaneously with the brain slices to convert the DLU/mm² values to Bq/mm². 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-HT₁A receptors (Hall et al. 1997).

**Microdialysis experiments**

Preceding surgery, rats were anesthetized by means of isoflurane 2%, 600 ml/min O₂ and 400 ml/min N₂O. 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 to 48 hours 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, CaCl₂ 1.2, and MgCl₂ 1.2, at a flow-rate of 1.5 l/m). 15-minute microdialysis samples 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 [¹⁸F]-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 minutes post injection. 5-HT levels were maximal at 60 minutes 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 5 fold increase in the raphe nucleus. Peak levels were achieved 45 minutes after administration (Figure 1).

**Ex vivo autoradiography**

**[^18F]-MPPF distribution**

After administration of[^18F]-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 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. 2000b; Shiue et al. 1997). Region over cerebellum ratios ranged from approximately 1 in the striatum to around 10 in the dorsal raphe nucleus.
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.
Effect of fenfluramine pretreatment

Administration of fenfluramine did not significantly affect cerebellar $[^{18}\text{F}]-$MPPF binding, compared to control rats. In fenfluramine treated rats mean (± SD) radioactivity in the cerebellum was 0.028 (±0.017) %ID, compared to 0.030 (±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 $[^{18}\text{F}]-$MPPF binding is reduced in the fenfluramine treated rat compared to the control rat. In table 1 and figure 3 the mean $[^{18}\text{F}]-$MPPF binding values (ratio to cerebellum) of control and fenfluramine treated rats are shown. Fenfluramine treatment resulted in a significant reduction in $[^{18}\text{F}]-$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.

Figure 2: Ex vivo phosphor screen images, 30 minutes after i.v. injection of $[^{18}\text{F}]-$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.
**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 (± SD) radioactivity in the cerebellum was 0.029 (±0.006) %ID, compared to 0.030 (±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.

![Figure 3](image-url)

**Figure 3:** Mean (± SD) regional ex-vivo $[^{18}F]$-MPPF binding (ratio to cerebellum), 30 minutes after i.v. injection of the ligand to conscious rats. $[^{18}F]$-MPPF was administered 30 minutes 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.
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 $^{18}$F-MPPF injection and were still present at the moment of decapitation.

Table I: Mean ($\pm$ SD) regional ex-vivo $^{18}$F-MPPF binding (ratio to cerebellum), 30 minutes after i.v. injection of the ligand to conscious rats. % 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).

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (n=7) Mean ± SD</th>
<th>Fenfluramine (n=7) Mean ± SD (% change)</th>
<th>Ketanserin + citalopram (n=5) Mean ± SD (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal ctx</td>
<td>4.0 ± 0.3</td>
<td>3.0 ± 0.3 (-23%) **</td>
<td>4.1 ± 0.4 (+3%)</td>
</tr>
<tr>
<td>Cingulate ctx</td>
<td>3.4 ± 0.5</td>
<td>2.9 ± 0.4 (-12%)</td>
<td>3.5 ± 0.4 (+4%)</td>
</tr>
<tr>
<td>Septum</td>
<td>9.7 ± 1.7</td>
<td>8.4 ± 0.6 (-13%)</td>
<td>10.1 ± 1.5 (+4%)</td>
</tr>
<tr>
<td>Striatum</td>
<td>1.1 ± 0.1</td>
<td>1.0 ± 0.1 (-5%)</td>
<td>1.0 ± 0.1 (-3%)</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.3 ± 0.1</td>
<td>1.1 ± 0.1 (-14%)</td>
<td>1.3 ± 0.1 (+5%)</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>2.5 ± 0.4</td>
<td>1.7 ± 0.4 (-33%) *</td>
<td>2.5 ± 0.6 (-1%)</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>8.3 ± 1.3</td>
<td>6.7 ± 0.4 (-19%)</td>
<td>8.3 ± 1.0 (-0%)</td>
</tr>
<tr>
<td>Amygdala</td>
<td>4.8 ± 0.3</td>
<td>3.6 ± 0.4 (-24%) **</td>
<td>4.6 ± 0.9 (-3%)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>8.8 ± 1.0</td>
<td>7.1 ± 0.7 (-20%) *</td>
<td>8.8 ± 1.2 (-0%)</td>
</tr>
<tr>
<td>Interpedunc ncl.</td>
<td>6.2 ± 1.2</td>
<td>5.8 ± 0.4 (-7%)</td>
<td>6.4 ± 1.1 (+3%)</td>
</tr>
<tr>
<td>Dorsal raphe</td>
<td>10.3 ± 1.3</td>
<td>7.8 ± 1.8 (-24%)</td>
<td>8.5 ± 0.7 (-17%)</td>
</tr>
<tr>
<td>Median raphe</td>
<td>3.9 ± 0.6</td>
<td>3.0 ± 0.5 (-23)</td>
<td>3.6 ± 0.6 (-7%)</td>
</tr>
</tbody>
</table>
Discussion

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

Previous ex vivo dissection studies in non-anesthetized and anesthetized animals (Hume et al. 2001; Maeda et al. 2001, respectively) did not show a significant effect of fenfluramine on the distribution of $[^{11}\text{C}]-\text{WAY-100635}$. However, in both studies, a non-significant 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 sacrificed 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 sacrificed before or after the peak in 5-HT response, respectively. Using PET, Hume et al. (2001) reported a 20% reduction in $[^{11}\text{C}]-\text{WAY-100635}$ 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). $[^{18}\text{F}]-\text{MPPF}$ binding was studied in anesthetized rats using a $\beta^+$ 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 $[^{18}\text{F}]-\text{MPPF}$ 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 $[^{11}\text{C}]-\text{WAY-100635}$, $[^{18}\text{F}]-\text{MPPF}$ has a much lower affinity for the 5-HT$_{1\text{A}}$ receptor (Ki of 0.8 nM and 3.3 nM, respectively) (Zhuang et al. 1994). According to Zimmer et al. (2002a), $[^{18}\text{F}]-\text{MPPF}$ 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
[18F]-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 [18F]-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 [18F]-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-HT1A receptor, however, this is not very likely since the affinity of fenfluramine for the 5-HT1A receptor is very low (μM range) (Mennini et al. 1991). Furthermore, the pharmacological treatments may have affected non-specific 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 [18F]-MPPF binding. However, if one assumes the reduction in [18F]-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-HT1A receptor availability, due to an increase in intrasynaptic 5-HT levels.

The binding of [18F]-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-HT1A receptors are located both within the synapse and extrasynaptically (Azmitia et al. 1996;
Riad et al. 2000). In a previous study using $[^{18}\text{F}]$-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 $K_i$ (Abi-Dargham et al. 1999):

$$B = \frac{(B_{max} \times L)}{K_D \left(1 + \frac{F_{5-HT}}{K_i}\right) + L}$$

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:

$$\frac{BP_2}{BP_1} = \frac{(1 + \frac{F_{5-HT1}}{K_i})}{(1 + \frac{F_{5-HT2}}{K_i})}$$

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 nM 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 artifact. 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 can not 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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References


