SYNAPSE 46:108–115 (2002)

5-HT_{1A} Receptor Imaging in the Human Brain: Effect of Tryptophan Depletion and Infusion on [^{18}F]MPPF Binding

JOANNA I. UDO DE HAES,[1*] FOKKO J. BOSKER,[1] AREN VAN WAARDE,[2] JAN PRUIM,[2] ANTOON T.M. WILLEMSEN,[2] WILLEM VAALBURG,[2] AND JOHAN A. DEN BOER[1]
[1]*Department of Biological Psychiatry, University Hospital Groningen, 9700 RB Groningen, The Netherlands*
[2]*PET Center, University Hospital Groningen, 9700 RB Groningen, The Netherlands*

KEY WORDS PET; 5-HT_{1A} receptor; serotonin; [^{18}F]MPPF; intrasynaptic; extrasynaptic; affinity

ABSTRACT The 5-HT_{1A} receptor has been implicated in a variety of physiological processes, psychiatric disorders, and neurodegenerative disorders. [^{18}F]MPPF is a useful radioligand for quantitative imaging of 5-HT_{1A} receptors in human subjects. Previous studies have shown that the binding of some radioligands is sensitive to changes in neurotransmitter concentration, whereas in other cases, binding is not affected. In the present study we investigated if [^{18}F]MPPF binding to the 5-HT_{1A} receptor is sensitive to changes in 5-HT. Changes in 5-HT levels were achieved by influencing its synthesis through tryptophan depletion, including a tryptophan-free amino acid drink 4.5 h prior to the PET scan and tryptophan infusion (10 mg/ml, 50 mg/kg, 30 min, starting 60 min prior to the PET scan). Binding of [^{18}F]MPPF in the brain of six healthy, male volunteers was compared in these two conditions. Mean binding potentials in the medial temporal cortex, cortical regions, and raphe nucleus did not significantly differ between the two conditions. The results of the study show that, under the experimental conditions used, [^{18}F]MPPF binding was not affected. It is hypothesized that the increases in 5-HT levels needed to produce a measurable effect on [^{18}F]MPPF binding would be significantly greater than that possible with tryptophan manipulation. Therefore, in pathological conditions, where such large increases in 5-HT levels are not expected, [^{18}F]MPPF seems a useful ligand to measure 5-HT_{1A} receptor distribution without the interference of endogenous 5-HT. **Synapse 46:108–115, 2002.**

INTRODUCTION

The 5-HT_{1A} receptor is distributed widely throughout the brain, with the highest densities in the hippocampal formation, neocortex, and raphe nucleus (Hall et al., 1997). The 5-HT_{1A} receptor is implicated in a variety of physiological processes (Barnes and Sharp, 1999) such as sexual behavior, sleep, learning and memory, and homeostatic mechanisms. Several studies also report the possible involvement of this receptor in psychiatric and neurodegenerative disorders (Bantick et al., 2001; Hjorth et al., 2000; Oosterink et al., 1998).

The study of receptors in the living human brain has become possible by means of positron emission tomography (PET). Recently, two independent PET studies reported a decrease of 5-HT_{1A} receptor binding in depressed patients, using the radioligand [^{11}C]WAY-100635, a selective 5-HT_{1A} receptor antagonist (Drevets et al., 1999; Sargent et al., 2000). [^{18}F]MPPF (4-(2′-methoxyphenyl)-1-[2′-(*N*-2″-pyridinyl)-*p*-[^{18}F]fluorobenzamido]-ethylpiperazine) is another selective 5-HT_{1A} receptor antagonist. It can be prepared in high yields and due to the relatively long half-life of ^{18}F, this radioligand can be distributed to PET centers that do not have access to onsite radiotracer production facilities (Passchier et al., 2001). Previous studies in animals and healthy volunteers have shown that [^{18}F]MPPF displays a regional radioactivity uptake in good correlation with known 5-HT_{1A} receptor distribution and density (Gino-

*Correspondence to: Joanna I. Udo de Haes, Department of Biological Psychiatry, University Hospital Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands. E-mail: j.udo.de.haes@acggn.azg.nl

Received 11 December 2001; Accepted 2 July 2002

DOI 10.1002/syn.10134

vart et al., 2000; Le Bars et al., 1998; Passchier et al., 2000, 2001; Plenevaux et al., 2000; Shiue et al., 1997).

For an optimal use of [18F]MPPF in clinical studies, it is important to investigate whether the binding of the ligand is sensitive to changes in extracellular serotonin (5-hydroxytryptamine, 5-HT) concentration. Previous studies have shown that the binding of some radioligands is sensitive to changes in neurotransmitter concentration, whereas in other cases binding is not affected (Laruelle, 2000). The binding of the D_2 receptor ligand [11C]raclopride, for instance, is found to be sensitive to changes in dopamine release. The binding is decreased by elevation of extracellular dopamine concentration and increased after a reduction of dopamine levels (Ginovart et al., 1997; Volkow et al., 1994). These alterations in ligand binding are interpreted as changes in availability of the receptor after neurotransmitter release.

It has been hypothesized that for a tracer to be sensitive to changes in neurotransmitter concentration, it should reversibly bind to the receptor and rapidly clear from tissue to blood (Endres and Carson, 1998). Because [18F]MPPF dissociates relatively fast from the receptor, the binding could be sensitive to changes in brain 5-HT (Passchier et al., 2000).

If [18F]MPPF appears to be sensitive to changes in 5-HT levels, it may be used to measure alterations of neurotransmitter release associated with psychiatric disorders such as depression (Blier and de-Montigny, 1994). On the other hand, when changes in neurotransmitter levels do not affect [18F]MPPF binding, the ligand is suitable to detect differences in receptor distribution irrespective of endogenous 5-HT levels.

In human subjects, the effect of changes in brain 5-HT levels can be studied by varying plasma tryptophan levels. Tryptophan is the precursor of 5-HT. Because brain 5-HT synthesis depends on the availability of this precursor, changes in plasma tryptophan lead to dose-related alterations in brain 5-HT levels and 5-HT turnover (Fernstrom and Wurtman, 1971; Moja et al., 1989). A decrease in tryptophan levels can be induced by tryptophan depletion, consisting of a low-tryptophan diet followed by a tryptophan-free amino acid drink (Delgado et al., 1990; Young et al., 1985). A reduction in total and free plasma tryptophan levels of around 90% is achieved within 5 h after administration of the amino acid drink (Delgado et al., 1990). Moreover, studies in healthy volunteers show that cerebrospinal fluid (CSF) levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) are decreased after tryptophan depletion, indicating that 5-HT function is indeed reduced by this method (Carpenter et al., 1998; Williams et al., 1999). Plasma tryptophan levels can be increased by means of tryptophan infusion. A study by Young and Gauthier (1981) in human volunteers showed that CSF 5-HIAA levels increased after tryptophan administration, indicating increased 5-HT synthesis, release, and metabolism. In addition, microdialysis studies in rats showed that 5-HT levels in the brain are indeed affected by changes in plasma tryptophan levels (Stancampiano et al., 1997; Westerink and de Vries, 1991).

In the present study, the sensitivity of [18F]MPPF binding to changes in 5-HT in human subjects was investigated by comparing [18F]MPPF binding potential (BP) after tryptophan depletion and tryptophan infusion. In order to induce maximal differences in receptor availability, PET scans were performed in both conditions in the same subject.

MATERIALS AND METHODS

General design

Binding of [18F]MPPF was compared in two conditions, separated by 7–10 days. In one condition, 5-HT synthesis was decreased by tryptophan depletion consisting of a low-tryptophan diet and a tryptophan-free amino acid drink 4.5 h before the PET scan (see below). In the other condition, 5-HT synthesis was increased by tryptophan infusion, starting at 60 min before the PET scan (see below). Each volunteer was studied in both conditions, thereby eliminating interindividual variation. The sequence of the tryptophan depletion and infusion condition was randomly assigned. The starting time of all [18F]MPPF PET scans was between 13:30 and 16:00. Blood samples were taken for tryptophan analyses and the profile of mood states (POMS) (McNair et al., 1971) was used to record adverse reactions. Subjects were asked to refrain from alcohol use on the evening before the PET scan and from caffeine-containing products on the day of the PET scan. After the PET scan, food and drink intake was no longer restricted.

Subjects

The study was approved by the medical ethics committee of the Groningen University Hospital. Six healthy male volunteers were included (age range: 19–62 years) after written informed consent had been obtained. Only male volunteers were included to avoid possible effects of the menstrual cycle on 5-HT_{1A} receptor density. Suitability to participate in the study was determined by an independent physician after a medical examination including an ECG and blood laboratory tests. The subjects were nonsmoking, without history of neurodegenerative or psychiatric disorders, and did not use psychoactive drugs or corticosteroids.

Radiochemistry

[18F]MPPF was prepared by nucleophilic [18F] fluorination of the appropriate nitro precursor (see Shiue et al., 1997, for a comparable method). Thereafter, it

was formulated into saline containing 10% ethanol. 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.

PET protocol

The PET scans were performed in 3D acquisition mode using a Siemens ECAT Exact HR+ camera, giving 63 slices with a center-to-center distance of 2.425 mm. Before the start of the PET scan the volunteers were positioned in the camera and their heads were fixed using a head restraint. A transmission scan was obtained using ^{68}Ge/^{68}Ga-rod-sources for attenuation correction of the dynamic PET scan. After the transmission scan, [^{18}F]MPPF (110–160 MBq) was administered as a 60-sec injection in a vein in one of the lower forearms. Twenty-one consecutive frames were acquired over a period of 60 min. [^{15}O]H_2O (500 MBq) was injected to assess regional cerebral blood flow. Data were acquired over a period of 3.5 min.

Regions of interest

The image obtained from the [^{15}O]H_2O scan was used to obtain anatomical information to draw regions of interest (ROIs). The regions were manually drawn in the transaxial orientation using a contour tool (Clinical Applications Programming Package; Siemens/CTI, Knoxville, TN). The areas were located using a stereotaxic atlas (Talairach and Tournoux, 1989). ROIs were defined for the medial temporal cortex (MTC) including the hippocampus, frontal cortex, cingulate cortex, insular cortex, lateral temporal cortex, raphe nucleus, and cerebellum. The ROI for the raphe nucleus was directly drawn on the BP image (see below). Each ROI was drawn in three (MTC, raphe nucleus, and cerebellum) or five (all other areas) consecutive planes and the data were pooled over the planes so that the mean value for the entire region was obtained. Right and left regions of bilateral ROIs were averaged.

Binding potential calculation

BPs were calculated using a simplified reference tissue model on a pixel-by-pixel basis, with the cerebellum as reference region (Gunn et al., 1997).

The same ROI boundaries were used to analyze the two scans of each subject after realignment of the two [^{18}F]MPPF BP images to the corresponding [^{15}O]H_2O scan by means of statistical parametric mapping (SPM 99) (Frackowiak et al., 1997).

Tryptophan depletion

Tryptophan depletion consisted of a 24-h low-tryptophan diet (diet available on request) followed the next morning by a tryptophan-free amino acid drink in fasting condition. The amino acid drink was composed of 15 amino acids as described by Young et al. (1985). Just before administration, 300 ml cooled tap water was added and the mixture was flavored with artificial fruit flavor. The drink was administered approximately 4.5 h before the [^{18}F]MPPF PET scan and consumed over a period of 20 min. The subjects remained in a quiet room during the following hours. They were allowed to drink water but could not eat food or take other drinks until the end of the PET scan. Approximately 1 h before start of the PET scan, the subjects were transported to the PET center. Blood samples for tryptophan determinations were taken through an indwelling intravenous catheter just before administration of the amino acid drink (t = −270 min), and 180 min (t = −180), 120 min (t = −120) and 60 min (t = −60) before the PET scan, at the start of the [^{18}F]MPPF PET scan (t = 0), and at the end of the [^{18}F]MPPF PET scan (t = 60). The samples were immediately placed on ice. POMS rating scales were completed just prior to plasma sampling.

Tryptophan infusion

Tryptophan infusions were prepared by dissolving 10 g of tryptophan in 1,000 ml of a 0.74% NaCl solution with NaOH added to bring the solution to a pH level of 7.3–7.5. The solution was sterilized by passage through a 0.2 μm filter. The solution was tested for pyrogenicity and sterility before use. A dose of 50 mg/kg tryptophan was infused over 30 min through an indwelling i.v. catheter, starting 60 min before the [^{18}F]MPPF PET scan. Blood samples for tryptophan determinations were taken just prior to the start of the infusion (t = −60), at the end of the infusion (t = −30), at the start of the [^{18}F]MPPF PET scan (t = 0), 30 min after start of the PET scan (t = 30), and at the end of the PET scan (t = 60). The samples were immediately placed on ice. POMS rating scales were completed just prior to plasma sampling.

Tryptophan analysis

Free tryptophan was isolated using the method of Eynard et al. (1993). Free and total tryptophan levels were then determined by high-performance liquid chromatography (Kema et al., 2001).

Statistical analyses

BPs in the two conditions were compared using the Wilcoxon signed rank test. Spearman correlation's were used to assess if BP changes between the two conditions were correlated to changes in cerebellar activity (AUC in cerebellum corrected for injected radioactivity), age, or sequence of experimental condition.

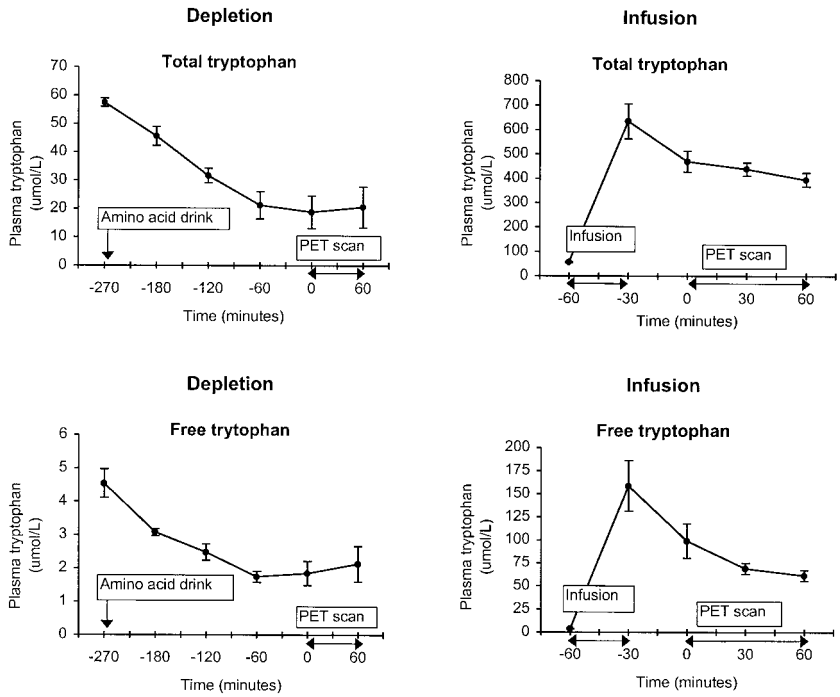

Fig. 1. Mean (SEM) plasma tryptophan levels (total and free) after tryptophan depletion and infusion.

RESULTS

Tryptophan challenges

Tryptophan depletion

The amino acid drink was consumed completely by five of the volunteers. Volunteer 3 could only drink one-third of the mixture because of the unpalatable taste. Mean plasma total and free tryptophan levels of all six subjects were reduced compared to baseline levels (67% and 62%, respectively) (Fig. 1). The plasma total and free tryptophan levels of the subject who did not consume the whole drink were reduced by 30% and 48%, respectively. Approximately 1–1.5 h after intake of the amino acid drink some of the subjects experienced nausea, abdominal fullness, and light drowsiness, as shown by the POMS ratings. No significant changes in mood were picked up by the POMS.

Tryptophan infusion

Tryptophan infusion caused an increase in total and free tryptophan levels compared to baseline (1,021% and 3,986% respectively) (Fig. 1). Some of the volunteers reported slight dizziness after the infusion, as shown by the POMS ratings.

[18F]MPPF binding

Distribution

After administration of [18F]MPPF, the distribution of radioactivity was in agreement with previous results and with known 5-HT_{1A} receptor localization, with the highest uptake in the MTC and low uptake in the cerebellum (Ginovart et al., 2000; Le Bars et al., 1998; Passchier et al., 2000, 2001; Plenevaux et al., 2000; Shiue et al., 1997).

Binding potentials in the two conditions

BPs were calculated for the MTC, frontal cortex, cingulate cortex, insular cortex, lateral temporal cortex, and raphe nucleus. Significant differences between the two conditions were not observed for the mean BPs in the various regions (Fig. 2). The mean BP changes in the combined cortical regions were in the same direction as changes in the individual cortical areas. Therefore, for further analysis, BP data for the frontal cortex, cingulate cortex, insular cortex, and lateral temporal cortex were combined. After tryptophan infusion, compared to tryptophan depletion, the mean change in BP was −0.6% in the MTC (range −14% to +16.6%), +1.9% in the cortical regions (range −11.6% to +22.1%) and −16.6% in the raphe nucleus (range −75.3 to +37.0%) (Table I).

There was no correlation between changes in BP in the different regions and changes in cerebellar radioactivity, age, or sequence of condition.

DISCUSSION

The aim of this study was to investigate if [18F]MPPF binding to the 5-HT_{1A} receptor is sensitive to changes in 5-HT concentration in human subjects. Therefore, [18F]MPPF binding was investigated after tryptophan depletion and tryptophan infusion, since changes in plasma tryptophan levels have been found to induce changes in brain 5-HT levels (Fernstrom and Wurt-

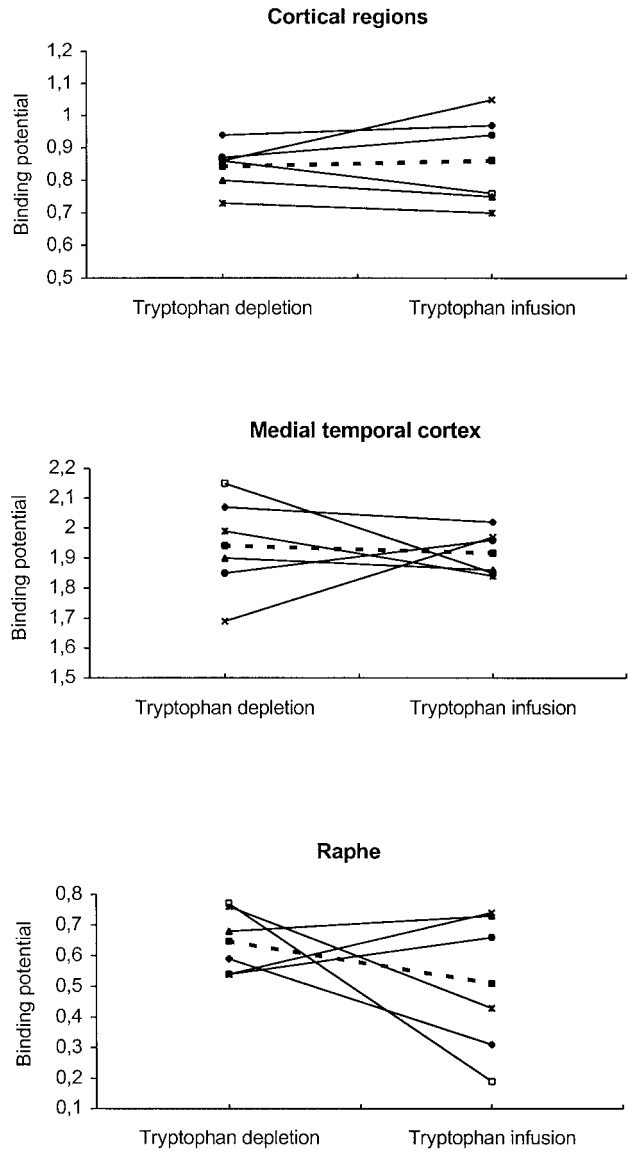

Fig. 2. Individual and mean (dotted line) BPs after tryptophan depletion and tryptophan infusion in the cortical regions, medial temporal cortex, and raphe.

man, 1971; Moja et al., 1989). The results of the study show that, under these experimental conditions, [^{18}F]MPPF binding was not affected by the changes in 5-HT concentration. In the raphe nucleus the mean BP decreased by 16.6% after tryptophan infusion, which could be interpreted as competition between [^{18}F]MPPF and 5-HT. However, when looking at the data more carefully, it appears that a decrease is only seen in half of the subjects. In the other three subjects, BPs were slightly increased after tryptophan infusion compared to tryptophan depletion. Moreover, because of the large variability in BPs, probably caused by the small size of the raphe nucleus, it is very hard to draw any conclusions from these data.

TABLE I. Individual and mean BPs after tryptophan depletion and infusion for the combined cortical areas, medial temporal cortex (MTC) and raphe nucleus

Volunteer	Region	Depletion	Infusion	% Change
1	Cortical areas	0.94	0.97	+3.2
	MTC	2.07	2.02	−2.4
	Raphe	0.59	0.31	−47.5
2	Cortical areas	0.86	0.76	−11.6
	MTC	2.15	1.85	−14.0
	Raphe	0.77	0.19	−75.3
3	Cortical areas	0.80	0.75	−6.3
	MTC	1.90	1.86	−2.1
	Raphe	0.68	0.73	+7.4
4	Cortical areas	0.86	1.05	+22.1
	MTC	1.69	1.97	+16.6
	Raphe	0.54	0.74	+37.0
5	Cortical areas	0.73	0.70	−4.1
	MTC	1.99	1.84	−7.5
	Raphe	0.76	0.43	−43.4
6	Cortical areas	0.87	0.94	+8.1
	MTC	1.85	1.96	+5.9
	Raphe	0.54	0.66	+22.2
Mean (SD)	Cortical areas	0.84 (0.07)	0.86 (0.14)	+1.9 (12.1)
	MTC	1.94 (0.17)	1.92 (0.08)	−0.6 (10.7)
	Raphe	0.65 (0.11)	0.51 (0.23)	−16.6 (44.9)

Recently, a few PET studies were performed in which the sensitivity of 5-HT ligands to changes in 5-HT levels was investigated. Using [^{18}F]altanserin, it was shown that ligand binding to the 5-HT$_2$ receptor was decreased after administration of the 5-HT reuptake inhibitor clomipramine (Larisch et al., 2000). Studies using 5-HT$_{1A}$ receptor radioligands, however, did not show consistent results. After administration of the 5-HT releaser and reuptake inhibitor fenfluramine (10 mg/kg i.p.), a decrease in [^{11}C]WAY-100635 BP was seen in the hippocampus of the rat, but not in the prefrontal cortex or raphe nucleus (Hume et al., 2001). In contrast, using the same radioligand, Maeda et al. (2001) did not find an effect of fenfluramine (10 mg/kg i.p.) in the hippocampus of the rat. Recently, a study was performed using a β^+ radiosensitive probe to investigate changes in [^{18}F]MPPF binding in vivo after different doses of fenfluramine. The authors reported a dose-related decrease of [^{18}F]MPPF binding in the hippocampus of the rat (Zimmer et al., 2001). Rabiner et al. (2000) investigated the binding of [^{11}C]WAY-100635 in the prefrontal cortex and MTC of human volunteers after either tryptophan depletion or tryptophan infusion compared to baseline. They did not find consistent changes in the expected direction after these challenges.

These discrepancies may be related to differences in the timing and extent of the alterations in 5-HT levels. In the studies of Hume et al. (2001), Maeda et al. (2001), and Zimmer et al. (2001), changes in extracellular 5-HT were estimated via microdialysis. Hume et al. (2001) showed that fenfluramine treatment resulted in a 15-fold increase in extracellular 5-HT levels in the hippocampus, whereas the increase in the prefrontal cortex was only 5-fold. In the study of Zimmer et al. (2001), the maximum increase in 5-HT was also 15-

fold, whereas the maximum 5-HT increase at the time of the PET scan in the study of Maeda et al. (2001) was not more than 5-fold. Studies in rats indicate that decreases in extracellular 5-HT after tryptophan depletion, as used in the present study and the study of Rabiner et al. (2000), are only 50% (Stancampiano et al., 1997) and maximal increases in 5-HT after tryptophan infusion are only 2-fold (Wurtman and Fernstrom, 1975; Young and Gauthier, 1981). Thus far, the data from animal studies indicate that substantial increases in 5-HT are needed to measure changes in ligand binding to the 5-HT_{1A} receptor.

When exploring this issue in further detail, it is important to consider that the 5-HT_{1A} receptor can exist in a high- and low-affinity state (Gozlan et al., 1995; Khawaja, 1995; Mongeau et al., 1992; Nénonéné et al., 1994; Watson et al., 2000). The proportion of 5-HT_{1A} receptors in the high-affinity state may depend on the brain area examined (Khawaja, 1995). Antagonists, such as [18F]MPPF and [11C]WAY-100635 will bind with equal affinity to either state, whereas agonists such as 5-HT will preferentially bind to the high-affinity state (Gozlan et al., 1995; Khawaja, 1995; Mongeau et al., 1992; Nénonéné et al., 1994; Watson et al., 2000). It can be argued that changes in 5-HT will therefore mainly effect [18F]MPPF and [11C]WAY-100635 binding at the receptors in the high-affinity state. Consequently, changes in binding can only be detected when a large enough proportion of 5-HT_{1A} receptors is in the high agonist affinity state or, alternatively, when 5-HT increases are sufficiently large to also affect ligand binding to receptors in the low agonist affinity state.

Of relevance in this respect could be that 5-HT_{1A} receptors are located both within the synapse and extrasynaptically (Azmitia et al., 1996; Riad et al., 2000,). It has been suggested that extrasynaptic 5-HT_{1A} receptors are activated by the low, ambient concentration of 5-HT, which is permanently maintained in the extracellular space, thereby enabling the widespread effects of 5-HT in global physiological processes and behavioral and therapeutic effects (Riad et al., 2000). Following this idea, regulation through extrasynaptic 5-HT_{1A} receptors is optimal when extracellular 5-HT levels are in the range of the Kd. The microdialysis studies by Hume et al. (2001) and Maeda et al. (2001) indicate that, even following fenfluramine administration, extracellular 5-HT levels are in the range of 6–35 nM, which is at least a factor of 10 below the affinity of 5-HT for the low agonist affinity state (Gozlan et al., 1995; Khawaja et al., 1995; Nénonéné et al., 1994; Watson et al., 2000). Taken together, we expect the major part of extracellular 5-HT_{1A} receptors to be in the high agonist affinity state. Can our results and those of the abovementioned studies, be explained in terms of competition at the extrasynaptic receptors? Using the law of mass action: $B = (B_{max}.[5\text{-HT}])/(Kd+[5\text{-HT}])$ and assuming a 5-HT concentration equal to the Kd, a 2-fold increase or decrease in 5-HT concentration, as used in the current study, would result in an increase or decrease of 5-HT occupancy from 50% of B_{max} to 67% or 33% of B_{max}, respectively. A 5-fold increase as found in the studies of Maeda et al. (2001) and Hume et al. (2001) would result in an increase in occupancy from 50% of B_{max} to 83% of B_{max}. Such changes seem large enough to induce changes in ligand binding. This is at variance with the present study, wherein we could not demonstrate significant changes in [18F]MPPF binding. A reasonable explanation could be that the proportion of extrasynaptic receptors is low and most 5-HT_{1A} receptors are thus located intrasynaptically.

Can our results be explained in terms of competition at intrasynaptic 5-HT_{1A} receptors? According to Maeda et al. (2001) and Hume et al. (2001), baseline 5-HT levels are considerably lower than those of dopamine. This may partly explain the differences in sensitivity to neurotransmitter changes between D_2 and 5-HT_{1A} ligands. Moreover, a large part of the 5-HT_{1A} receptors may be in the low agonist affinity state (Nénonéné et al., 1994). To measure competition at this receptor state, large increases in 5-HT may be necessary. Arguably, manipulation of 5-HT synthesis by tryptophan depletion or infusion may be too subtle to achieve this.

The question is, how to induce sufficiently large increases in 5-HT in human subjects? It can be argued that a decrease in BP in the current study may have been seen after a higher dose of tryptophan. However, previous experiments in rats and human subjects indicate that 5-HT synthesis is not further increased at tryptophan doses exceeding 50 mg/kg. In the present study tryptophan was infused over 30 min, starting 60 min prior to the PET scan. Rat experiments showed that brain levels of 5-HT are maximally increased within the first 30–60 min of tryptophan injection (Sharp et al., 1992).

Future studies in humans should explore other methods to increase 5-HT; for example, by interfering with its release and reuptake. In the animal studies of Zimmer et al. (2001) and Hume et al. (2001), detectable decreases in radioligand binding were seen after administration of fenfluramine. Fenfluramine both releases 5-HT and blocks the reuptake sites of 5-HT, which may increase 5-HT in the synapse to levels sufficiently high for competition at the low-affinity state. Unfortunately, safety considerations preclude the use of comparable fenfluramine doses in man. Perhaps strategies based on reuptake inhibition combined with autoreceptor blockade offer an alternative.

To summarize, the results of the present study with [18F]MPPF complement recent studies in rat and man, using [11C]WAY-100635, and suggest that under baseline conditions only a few percent of the intrasynaptic

5-HT_{1A} receptor population is occupied by 5-HT. In order to attain measurable competition between [^{18}F]MPPF and 5-HT, large increases in 5-HT are necessary.

Therefore, in pathological conditions, where such large increases in 5-HT levels are not expected, [^{18}F]MPPF seems a useful ligand to measure 5-HT_{1A} receptor binding potentials without interference of endogenous 5-HT.

ACKNOWLEDGMENTS

We thank the staff of the PET center for their support, Remge Pieterman for the medical examination, Peter Hulshof for medical support, Ido Kema and his team for tryptophan analysis, and Mrs. Kok for providing the low-tryptophan diet.

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