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RESEARCH PAPER

Characterization of 4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine (*p*-DMPPF) as a new potent 5-HT$_{1A}$ antagonist

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Background and purpose: The identification of potent and selective radioligands for the mapping of 5-HT receptors is interesting both for clinical and experimental research. The aim of this study was to compare the potency of a new putative 5-HT$_{1A}$ receptor antagonist, *p*-DMPPF, (4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine) with that of the well-known 5-HT$_{1A}$ antagonists, WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide) and its fluorobenzoyl analogue, *p*-MPPF (4-(2-methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine).
Experimental approach: Single cell extracellular recordings of dorsal raphe (DR) neurones were performed in rat brain slices. The potency of each compound at antagonizing the effect of the 5-HT$_{1A}$ agonist, 8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)-tetraline], was quantified using the Schild equation. The pharmacological profile of *p*-DMPPF was defined using competition binding assays.
Key results: Consistently with a 5-HT$_{1A}$ receptor antagonist profile, incubation of slices with an equimolar (10 nM) concentration of each compound markedly reduced the inhibitory effect of 8-OH-DPAT on the firing rate of DR neurones, causing a significant rightward shift in its concentration-response curve. The rank order of potency of the antagonists was WAY-100635 > *p*-DMPPF ⩾ *p*-MPPF. The sensitivity of DR neurones to the inhibitory effect of 8-OH-DPAT was found to be heterogeneous. The binding experiments demonstrated that *p*-DMPPF is highly selective for 5-HT$_{1A}$ receptors, with a K_i value of 7 nM on these receptors.
Conclusions and implications: The potency of the new compound, *p*-DMPPF, as a 5-HT$_{1A}$ antagonist is similar to that of *p*-MPPF in our electrophysiological assay. Its selectivity towards 5-HT$_{1A}$ receptors makes it a good candidate for clinical development.
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Keywords: brain slices; 5-HT$_{1A}$ receptors; 5-HT$_{1A}$ antagonists; dorsal raphe; *p*-DMPPF

Abbreviations: DR, dorsal raphe nucleus; *p*-DMPPF, (4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]-piperazine; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)-tetraline; *p*-MPPF, 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine); WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide

Introduction

The serotonin (5-hydroxytryptamine, 5-HT) system is an important neurotransmitter network that is involved in the modulation of various physiological functions such as thermoregulation, pain perception, cardiovascular control, aggressive and sexual behaviour, mood, appetite and the sleep–wake cycle (Frazer *et al.*, 1990; Hartig *et al.*, 1993; Hoyer *et al.*, 1994).

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Among the numerous (at least 14) subtypes of 5-HT receptors presently identified, the 5-HT$_{1A}$ receptor is especially interesting because of its involvement in the neurochemical mechanisms underlying anxiety and depression and their treatment (Fletcher *et al.*, 1993). During the last decade, several groups have been involved in the development of radioligands for the study of brain 5-HT$_{1A}$ receptors with positron emission tomography (PET). Various halogenated derivatives of the selective 5-HT$_{1A}$ antagonist WAY-100635 (Figure 1) have thus been developed (Zhuang *et al.*, 1993; Wilson *et al.*, 1996; Carson *et al.*, 2000). Among these, the *p*-fluorobenzoyl analogue of WAY-100635, 4-(2′-methoxyphenyl)-1-[2′-

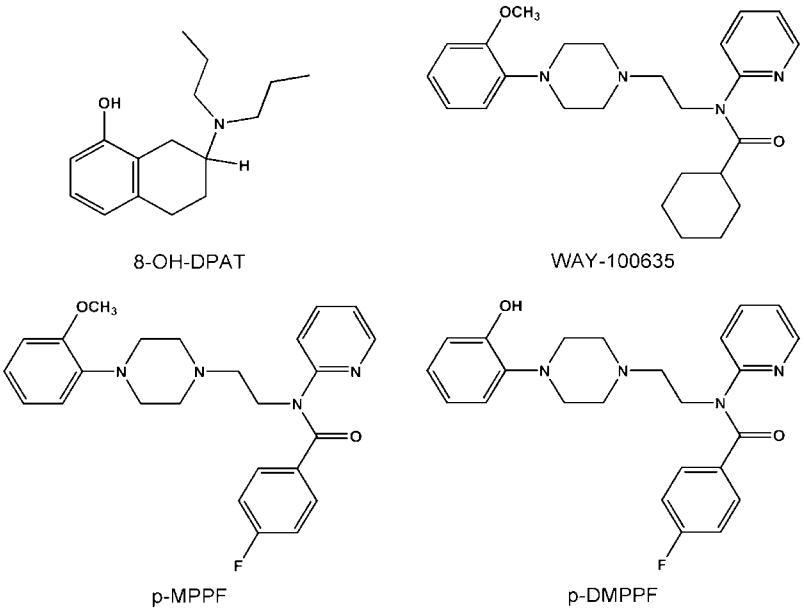

Figure 1 Structural formula of 8-hydroxy-2-(di-*n*-propylamino)-tetraline (8-OH-DPAT), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY-100635), 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine (*p*-MPPF) and 4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine (*p*-DMPPF).

[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine (*p*-MPPF; Figure 1), has been evaluated in both *in vitro* and *in vivo* models (Kung *et al*., 1996; Le Bars *et al*., 1998; Plenevaux *et al*., 2000) and it was found that this compound is a specific and selective antagonist at 5HT$_{1A}$ receptors and that *p*-[^{18}F]MPPF could represent a potentially interesting radiopharmaceutical for PET imaging of 5-HT neurotransmission. In contrast, it has been shown that DWAY, the desmethylated analogue of WAY100635 (Pike *et al*., 1998), gives a significantly higher radioactivity signal than its parent compound, providing improved imaging statistics and advantages in biomathematic modelling (Andree *et al*., 2002). In light of this information, it appeared interesting to undertake the evaluation of (4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine (*p*-DMPPF; Figure 1), the desmethylated derivative of *p*-MPPF. This compound was therefore synthesized in our laboratory, labelled with fluorine-18, and evaluated for *ex vivo* tissue distribution in rats. Tissue distribution and specificity of this compound were in total agreement with the known localization of 5-HT$_{1A}$ receptors in rats (Defraiteur *et al*., 2006).

Since there have been no comparative evaluations of the potencies of *p*-MPPF and *p*-DMPPF as antagonists at 5-HT$_{1A}$ receptors, we chose to examine their effect on the firing rate of presumed 5-hydroxytryptaminergic neurones of the dorsal raphe nucleus (DR) recorded extracellularly in rat brain slices. The firing of DR 5-hydroxytryptaminergic neurones has been shown to be inhibited by 5-HT$_{1A}$ receptor agonists such as 8-hydroxy-2-(di-*n*-propylamino)-tetraline (8-OH-DPAT; Rogawski and Aghajanian, 1981; Sprouse and Aghajanian, 1987; Aghajanian *et al*., 1990) and this effect is blocked by 5-HT$_{1A}$ antagonists (Forster *et al*., 1995). The inhibition of the firing of 5-hydroxytryptaminergic neurones by 5-HT$_{1A}$ agonists is mediated by the direct activation of 5HT$_{1A}$ somatodendritic receptors (Hamon *et al*., 1990) and the subsequent opening of G protein-coupled inwardly rectifying potassium channels (Williams *et al*., 1988). However, the neurobiology of the DR may be more complicated than what was first believed. Indeed, it has been recently shown that the DR nucleus contains both 5-hydroxytryptaminergic and non-5-hydroxytryptaminergic neurones, including GABAergic and catecholaminergic neurones (Allers and Sharp, 2003; Day *et al*., 2004; Lu *et al*., 2006). Moreover, directly identified 5-hydroxytryptaminergic and non-5-hydroxytryptaminergic DR neurones both respond to 5-HT$_{1A}$ agonists, although with a significantly smaller response for the non-5-hydroxytryptaminergic cells (Beck *et al*., 2004).

The aims of this study were (1) to investigate the effect of *p*-MPPF and *p*-DMPPF on the firing rate of DR neurones to assess whether these ligands have any partial agonist activity, in which case they should at least partially inhibit the firing of DR neurones by themselves, (2) to quantify their potency, as well as the one of the reference compound WAY-100635, in shifting the concentration-response curve to 8-OH-DPAT and (3) to establish the pharmacological profile of *p*-DMPPF.

Methods

Animals

Male Wistar rats weighing 150–200 g were used. All experiments were conducted according to the guidelines of the National Institute of Health (NIH publication no. 80–23, revised 1978) and were accepted by the Ethics Committee for the use of animals in Research of the University of Liege (protocol 383).

In vitro *electrophysiology*

Animals were deeply anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and given pure oxygen to breathe for 5 min. After decapitation, the brain was quickly removed and

cooled in ice-cold artificial cerebrospinal fluid (ACSF) of the following composition: 130 mM NaCl; 5 mM KCl; 24 mM $NaHCO_3$; 1.25 mM NaH_2PO_4; 10 mM D-glucose; 2 mM $CaCl_2$; and 1.25 mM $MgSO_4$. A piece of brainstem was prepared and cut in transverse sections by means of a vibratome. The thickness of the slices was about 400 μm. A slice containing the DR area was placed on a nylon mesh in a recording chamber (volume 0.5 ml). Since this structure has a length of $\sim$1 mm in the anterior–posterior plane, we selected the slice containing the decussation of the superior cerebellar peduncles and also were careful to record from neurones located close to the midline. This procedure was used in order to study a well-defined population of cells and to avoid biases related to the topographical heterogeneity of the region (Day *et al*., 2004). The tissue was held in position by short pieces of platinum. The slice was completely immersed in a continuously flowing ($\approx$2 ml min^{-1}), heated ($\pm$35°C) solution of the same composition as indicated above.

Extracellular recordings were made using glass micropipettes filled with ACSF (impedance: 5–10 MΩ and tip diameter: $\sim$2 μm). Action potentials were amplified 1000 times by a homemade amplifier and displayed on a Tektronix oscilloscope. The signals were also introduced into an amplitude discriminator and counted every 10 s. In addition, signals were also recorded with the Spike2 software (Cambridge Electronic Design, Cambridge, UK) in a majority of experiments.

Presumed 5-hydroxytryptaminergic neurones were identified with electrophysiological and pharmacological criteria, as described previously (Seutin *et al*., 1990) and below. Thus, unlike the situation *in vivo* in which the majority of DR 5-hydroxytryptaminergic neurones are spontaneously active, most DR presumed 5-hydroxytryptaminergic neurones are silent in the slice preparation (Vandermaelen and Aghajanian, 1983). In the presence of 10 μM phenylephrine (PE), they fire at a rate of 0.4–3 spikes s^{-1} and are characterized by long duration (>2 ms), often triphasic action potentials. Their firing is inhibited by 90–100% when PE is washed out. The firing is also inhibited by nanomolar concentrations of 8-OH-DPAT (see results). Taken together, these characteristics are extremely suggestive of the 5-hydroxytryptaminergic nature of the recorded neuron and at least allow the possibility of recording from GABAergic neurons (Allers and Sharp, 2003) to be excluded.

In all experiments, a 5-min control period was used to assess the stability of the firing rate. Drugs were superfused using three-way taps so that the flow remained constant. Each concentration was superfused until equilibrium was obtained (usually 10 min). When antagonists (WAY-100635, *p*-MPPF and *p*-DMPPF) were used, they were first superfused alone for 10 min to reach a steady-state concentration in the tissue before the application of the agonist.

Data analysis and statistical evaluation of electrophysiological study

EC_{50} determination: Four to seven increasing concentrations of 8-OH-DPAT in the range of 0.1–100 nM were superfused. For each concentration, the percentage inhibition relative to the mean control period firing rate was calculated and the concentration producing a 50% inhibition (EC_{50}) was graphically determined for each cell by extrapolation on a semi-logarithmic graph, where x is the concentration of the ligand and y the percentage inhibition (Bowery *et al*., 1994; Figures 2a and d).

pK_B determination: The ability of *p*-MPPF, *p*-DMPPF and WAY-100635 to displace the concentration–response curve of 8-OH-DPAT was evaluated. For this purpose, neurones were exposed to 8-OH-DPAT (range: 3–300 nM) in the presence of the antagonists (Figures 2b and c). A concentration of 10 nM was chosen for each antagonist. In preliminary experiments, this concentration was found to provide an effect that was significant, but also compatible with the use of reasonable concentrations of the agonist. The K_B of the tested ligands was then calculated using the Schild equation:

$$r - 1 = [B]/K_B$$

where r is the ratio between the EC_{50} of the agonist in the presence and absence of the antagonist and B the concentration of the competitive antagonist. Taking the negative logarithm yielded the pK_B.

Since values of EC_{50} in all groups were not distributed normally ($P<0.01$ for the four groups, Lilliefors test), values are expressed as medians$\pm$95% confidence interval (CI95). Statistical analysis of these data was performed using the Kruskal–Wallis test. Differences were considered significant when $P<0.05$. All other values are expressed as means$\pm$s.e.mean.

Drugs used for electrophysiology

p-MPPF and *p*-DMPPF were synthesized in our laboratory according to methods described previously (Zhuang *et al*., 1994). WAY-100635 and 8-OH-DPAT hydrobromide were purchased from Sigma-Aldrich (St Louis, MO, USA) and Tocris Bioscience (Bristol, UK), respectively. For electrophysiological experiments, stock solutions of *p*-MPPF and *p*-DMPPF were prepared in dimethylsulphoxide (DMSO). The final concentration of DMSO never exceeded 1%. Control experiments showed that this concentration of DMSO had no effect on the firing rate of the cells or their sensitivity to 8-OH-DPAT. Stock solutions of other compounds were prepared in water.

Binding studies

Competition experiments were performed at Cerep (Celle l'Evescault, France), using standard methods, on human receptors in transfected CHO cells, except in the case of α_1 and α_2 receptors, the binding to which was measured in rat cerebral cortex membranes. Steady-state binding of specific radioactive ligands was measured by scintillation counting. In all cases, nonspecific binding was measured using an excess concentration (500- to 10^5-fold) of an unlabelled ligand. Competitive binding of a reference ligand was performed for each receptor, which was studied and yielded results that were in agreement with published data.

All experiments using *p*-DMPPF were performed in duplicate. In a first set of experiments, displacement of specific

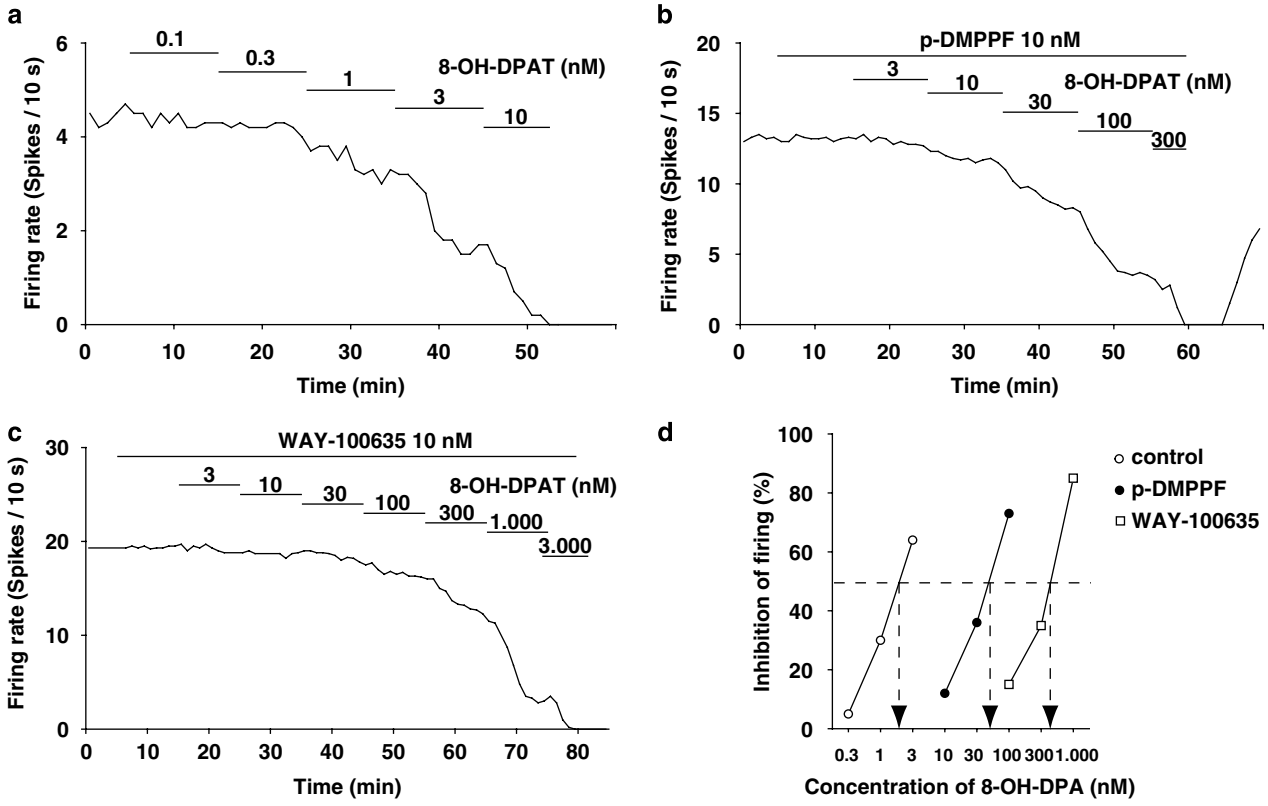

Figure 2 *In vitro* extracellular recording of three dorsal raphe nucleus (DR) neurones. (**a**) Increasing concentrations of 8-hydroxy-2-(di-*n*-propylamino)-tetraline (8-OH-DPAT) were superfused. (**b** and **c**) 8-OH-DPAT was applied in the continuous presence of 4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine (*p*-DMPPF) and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)-cyclohexanecarboxamide (WAY-100635), respectively. Note that higher concentrations of the agonist are needed to inhibit cell firing in the presence of the antagonists. (**d**) Plot showing the method used to calculate the EC_{50} in the above experiments when 8-OH-DPAT was applied alone, in the presence of *p*-DMPPF or of WAY-100635.

binding of radioactive ligands by 1 μM *p*-DMPPF was measured. When the displacement was found to be above 50%, IC_{50} and K_i values were determined.

IC_{50} values (concentrations producing a half-maximal inhibition of control-specific binding) and Hill coefficients (n_H) were determined by nonlinear regression analysis of the competition curves generated with mean replicate values using curve fitting to the Hill equation $(Y = D + [(A - D)/(1 + (C/C_{50})^{n_H})]$, where Y is the specific binding, D the minimum specific binding, A the maximum specific binding, C the compound concentration, $C_{50} = IC_{50}$ and n_H the slope factor). The inhibition constant (K_i) was calculated using the Cheng–Prusoff equation: $(K_i = IC_{50}/(1 + (L/K_D))$, where L is concentration of radioligand in the assay and K_D the affinity of the radioligand for the receptor. All experiments on *p*-DMPPF were performed in duplicate.

Results

Electrophysiology

Application of low nanomolar concentrations of 8-OH-DPAT induced a progressive inhibition of the firing of all DR neurones that were tested (Figure 2a). A complete cessation of firing was obtained in all cases. The effect of the 5HT$_{1A}$ agonist was generally reversible. However, EC_{50} values of 8-OH-DPAT were rather heterogeneous and were not distributed normally. The median EC_{50} was 5.1 nM (CI95: 3.4–9.2 nM; $n = 26$). Globally, this value is consistent with the data from Sprouse (1991), who reported an EC_{50} of 7 ± 2 nM under similar experimental conditions. In our case, the EC_{50} values ranged from 1.5 to 19 nM (Figure 3). There was no correlation between electrophysiological parameters (control firing rate or action potential duration) and sensitivity to 8-OH-DPAT in our population of cells.

Prior administration of each of the putative 5-HT$_{1A}$ receptor antagonists, WAY-100635, *p*-MPPF and *p*-DMPPF (at a concentration of 10 nM), produced a marked rightward shift in the concentration–response curve of 8-OH-DPAT on DR neurones ($P < 0.001$, Kruskal–Wallis test; Figures 2b, c and 3). None of the three antagonists had any effect on cell firing by itself. The results are summarized in Table 1. Note that the EC_{50} values obtained in the presence of the antagonists were also not normally distributed, again suggesting a heterogeneity in the sensitivity of presumed 5-hydroxytryptaminergic neurones within a restricted region (see Methods) of the DR to 5HT$_{1A}$ agonists. Comparison of the EC_{50} values, using *post hoc* paired comparisons, showed that *p*-MPPF tended to be slightly less potent than WAY-100635 ($P = 0.05$), whereas no significant difference existed between the results

obtained with *p*-DMPPF and WAY-100635 ($P=0.17$). In contrast, the results obtained with *p*-DMPPF and *p*-MPPF were equivalent. Use of the median value of the EC_{50} allowed us to obtain estimates for the pK_B values of WAY-100635, *p*-DMPPF and *p*-MPPF, as shown in Table 1.

Binding study

To further evaluate the potential of *p*-DMPPF as a putative 5HT$_{1A}$ radioligand, we assessed its affinity for 19 receptors, as shown in Table 2. Of those receptors, the compound only exhibited moderate affinity for α_1 and 5HT$_7$ receptors ($\sim$50% displacement of the respective radioligand at 1 μM), in addition to a high affinity for 5HT$_{1A}$ receptors, as expected from the electrophysiological data. The latter affinity was further determined with a complete curve. The IC_{50} and K_i values were 11 and 7 nM, respectively. Thus, *p*-DMPPF has at least $\sim$100-fold more affinity for 5HT$_{1A}$ receptors than for any other receptor that was tested.

Discussion

This study represents the first comparative evaluation of a new series of 5-HT$_{1A}$ receptor antagonists, *p*-MPPF and *p*-DMPPF, in an *in vitro* assay of 5-HT$_{1A}$ somatodendritic autoreceptor activity. The antagonist potency of both compounds on the response of DR neurones to 8-OH-DPAT was evaluated and compared to that of the classical 5-HT$_{1A}$ antagonist, WAY-100635.

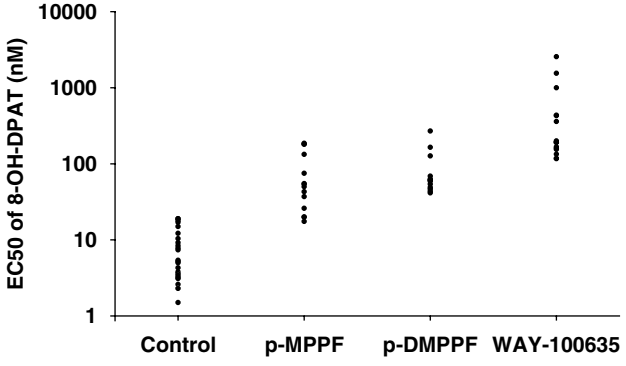

Figure 3 Summary plot of the EC_{50} values obtained under the various experimental conditions. Note that the EC_{50} values are shown on a log scale and hence the values are very dispersed in all groups.

Our electrophysiological experiments show that *p*-MPPF and *p*-DMPPF behave as competitive 5-HT$_{1A}$ receptor antagonists, causing a rightward shift in the concentration–response curve of 8-OH-DPAT, with no indication of any partial agonist effect. A similar effect was observed with WAY-100635, which is consistent with published data showing that this compound dose-dependently blocks the ability of 8-OH-DPAT to inhibit the firing of DR 5-HT neurones (Fletcher *et al*., 1996). Furthermore, a pK_B value close to 9.5 is in the range of values previously reported for this drug (Testa *et al*., 1999). Quantitatively, *p*-MPPF and *p*-DMPPF are slightly less potent antagonists than WAY-100635. No significant difference exists between *p*-MPPF and *p*-DMPPF, although the latter tends to be a slightly more potent 5-HT$_{1A}$ antagonist.

Our binding study further shows that *p*-DMPPF has a very good selectivity towards 5HT$_{1A}$ receptors since its affinity for these receptors is 100-fold greater than for α_1 and 5HT$_7$ receptors, and $>$100-fold greater than for 16 other monoamine receptors. These results are strongly suggestive of the ability of the compound to bind selectively to brain bold values signifier affinity of 5-HT$_{1A}$ receptors after systemic administration in humans, as also suggested by its tissue

Table 2 Percentage inhibition by 10^{-6} M *p*-DMPPF of the specific binding of the radioligand at various receptors

Receptor	*% inhibition of specific binding*
α_1 (nonselective)	47
α_2 (nonselective)	−2
β_1 *(h)*	4
D_1 *(h)*	−1
D_{2S} *(h)*	6
M_1 *(h)*	1
M_2 *(h)*	17
M_3 *(h)*	12
M_4 *(h)*	0
5-HT$_{1A}$	**100**
5-HT$_{1B}$	1
5-HT$_{2A}$	1
5-HT$_{2B}$	17
5-HT$_{2C}$	−3
5-HT$_3$	−3
5-HT$_{4e}$	−2
5-HT$_{5A}$	10
5-HT$_6$	0
5-HT$_7$	44

Abbreviation: *p*-DMPPF, 4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine.

Table 1 EC_{50} values of 8-OH-DPAT, alone or in the presence of 10 nM WAY-100635, *p*-MPPF or *p*-DMPPF in the electrophysiological assay in brain slices and deduced pK_B values

Drug	*Median EC_{50}*	*CI95 for EC_{50}*	r	*pK_B of antagonist*	n
8-OH-DPAT	5.1	3.4–9.2	—	—	26
8-OH-DPAT + WAY-100635 (10 nM)	191.5	155–435	37.6	9.57	16
8-OH-DPAT + *p*-MPPF (10 nM)	52	20–181	10.2	8.96	14
8-OH-DPAT + *p*-DMPPF (10 nM)	61	46.5–127	12.0	9.04	13

Abbreviations: *p*-DMPPF, 4-(2-hydroxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine; 8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)-tetraline; *p*-MPPF, 4-(2′-methoxyphenyl)-1-[2′-[*N*-(2″-pyridinyl)-*p*-fluorobenzamido]ethyl]piperazine; WAY-100635, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide.
Because values were not distributed normally, medians and CI95 values are given. Medians were used to calculate *r* values.

distribution in rats (Defraiteur *et al*., 2006). Moreover, the K_i value of *p*-DMPPF for 5HT$_{1A}$ receptors (7 nM) is not very different from its K_B value (0.9 nM) determined in our electrophysiological experiments. The lower K_B value might be due to the low level of coupling between the receptors and transducer proteins in slices in control conditions. This is known to increase the apparent affinity of antagonists in Schild analyses.

In the course of our study, we obtained evidence for a large heterogeneity in the sensitivity of presumed 5-hydroxytryptaminergic DR neurones to 8-OH-DPAT. This observation is consistent with numerous studies (Kirby *et al*., 2003; Beck *et al*., 2004; Day *et al*., 2004; Marinelli *et al*., 2004), which emphasize the complexity of the neuronal organization within the DR. According to Beck *et al*. (2004), identified 5-HT- and non-5HT-containing neurones in the DR nucleus have similar electrophysiological characteristics but differ in their response to 5-HT$_{1A}$ receptor stimulation, with a significantly smaller response of non-5-HT-containing neurones. Our results are consistent with this conclusion. In all our protocols, that is application of 8-OH-DPAT without or with any of the antagonists, a large variability to 5-HT$_{1A}$ receptor stimulation was found (up to a 20-fold range of EC_{50} in the presence of WAY-100635: 117–2560 nM). Taken together with previous studies, our results point to a significant heterogeneity of 5-HT$_{1A}$ sensitivity in the DR. If this heterogeneity exists at the level of the receptors themselves (for example, their surface density), this suggests that PET data may require analysis using a model that takes this heterogeneity into account. Another important topic will be to determine whether less sensitive neurones have another neurochemical (for example, catecholaminergic; Beck *et al*., 2004; Lu *et al*., 2006) phenotype or are simply 5-HT neurones that synthesize very little 5-HT.

In conclusion, we have confirmed the antagonist properties of WAY-100635 and *p*-MPPF in an assay *in vitro* of 5-HT$_{1A}$ receptor activity, and we have provided the first evidence that the newer compound, *p*-DMPPF, behaves similar to a 5-HT$_{1A}$ antagonist in this system. Indeed, *p*-DMPPF exhibits antagonist potency similar to that of the reference antagonist at 5-HT$_{1A}$ receptors, WAY-100635. Taken together with our demonstration of the selectivity of *p*-DMPPF for 5-HT$_{1A}$ versus other aminergic receptors, these data confirm the interest of this new agent as an additional radioligand for mapping 5-HT$_{1A}$ receptors in human experiments, as suggested by recently published data from our group (Defraiteur *et al*., 2006).

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Conflict of interest

The authors state no conflict of interest.

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