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In vitro profile of the antidepressant candidate OPC-14523 at rat and human 5-HT$_{1A}$ receptors

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Abstract

This study determined the in vitro functional profile of 1-[3-[4-(3-chlorophenyl)-1-piperazinyl]propyl]-5-methoxy-3,4-dihydro-2-quinolinone monomethanesulfonate (OPC-14523) at rat and human serotonin (5-HT) 5-HT$_{1A}$ receptors and binding affinity of OPC-14523 at human frontocortical 5-HT$_{1A}$ receptors. OPC-14523 (1 μM) increased guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding to 5-HT$_{1A}$ receptor-containing regions of rat brain tissue sections (~53% of the effect of 1 μM (+)8-hydroxy-2-(di-*n*-propylamino)tetralin ((+)8-OH-DPAT) that were blocked by the selective 5-HT$_{1A}$ receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100635). OPC-14523 also behaved as a partial agonist in its stimulation of [^{35}S]GTPγS binding to membranes from rat hippocampus (pEC$_{50}$=7.60±0.23, E_{max}=41.1% of the effect of 10 μM (+)8-OH-DPAT), human frontal cortex (pEC$_{50}$=7.89±0.08; E_{max}=64% of the effect of 10 μM (+)8-OH-DPAT), and Chinese Hamster Ovary cells expressing cloned human 5-HT$_{1A}$ receptors (pEC$_{50}$=8.0±0.11; E_{max}=85.5% of the effect of 10 μM 5-HT), and all of these effects of OPC-14523 were blocked by WAY-100635. Taken together, these data support the development of OPC-14523 as an antidepressant whose mechanism of action involves potent partial agonist activity at 5-HT$_{1A}$ receptors.

Keywords: OPC-14523; Depression; Serotonin

1. Introduction

The serotonin (5-HT) 5-HT$_{1A}$ receptor has been proposed as a therapeutic target for the development of improved antidepressant drugs (Hensler, 2003; Blier and Ward, 2003), based on the indication of azapirone 5-HT$_{1A}$ receptor partial agonists, including buspirone and gepirone, for the management of anxiety and depressive disorders (Robinson et al., 1990, 2003), and the ability of azapirones to elicit antidepressant-like responses in rodents (Wieland and Lucki, 1990). The antidepressant effects of 5-HT$_{1A}$ receptor agonists and serotonin selective reuptake inhibitors (SSRIs) have been tentatively linked to their desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors, which consequently enhance serotonergic neurotransmission to normo-sensitive 5-HT$_{1A}$ receptors, in regions such as the hippocampus and cortex, and subsensitive 5-HT$_{1A}$ receptors in other brain regions, such as the amygdala or hypothalamus (Hensler, 2003). The potential importance of 5-HT$_{1A}$ receptors as an antidepressant drug target is further implied by the ability of adjunctive (−)pindolol to shorten the onset to antidepressant therapy produced by SSRIs through its putative blockade of somatodendritic 5-HT$_{1A}$ autoreceptors (Zanardi et al., 1998; Perez et al., 2001).

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1-{3-[4-(3-chlorophenyl)-1-piperazinyl]propyl}-3,4-dihydro-5-methoxy-2(1*H*)-quinolinone monomethanesulfonate (OPC-14523), is a novel antidepressant drug candidate that binds with high affinity to rat 5-HT_{1A} receptors (IC_{50}=2.3 nM) and with moderate affinity (IC_{50}=47–56 nM) to rat σ_1 and σ_2 receptor subtypes (Oshiro et al., 2000; Tottori et al., 2001). A single oral administration of OPC-14523 produces antidepressant-like responses in rats and mice in the forced swimming test that are observed only after repeated treatment with the SSRI antidepressant fluoxetine. Interestingly, these effects of OPC-14523 are inhibited both by the selective 5-HT_{1A} receptor antagonist *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide (WAY-100635) and the σ receptor antagonist NE-100. The same study also showed that OPC-14523 induces flat body posture and inhibits forebrain 5-HT biosynthesis, providing additional evidence that OPC-14523 activates 5-HT_{1A} receptors in vivo. OPC-14523 also binds in vitro with a moderate affinity to the 5-HT transporter (IC_{50}=80 nM). While OPC-14523 dose-dependently inhibited 5-HT reuptake in rat synaptosomes ex vivo, OPC-14523 did not produce statistically significant reductions in 5-HT reuptake in vivo at doses below 100 mg/kg, p.o. (Tottori et al., 2001). Despite the availability of these data, which support the development of OPC-14523 as an antidepressant drug candidate, the in vitro functional characteristics of OPC-14523 at native or heterologously expressed 5-HT_{1A} receptors have not been described to date.

Guanosine-5′-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]GTPγS) binding assays have been used to measure functional activation of G-proteins by 5-HT_{1A} receptors expressed in rat (Sim et al., 1995; Alper and Nelson, 1998; Watson et al., 1998; Jordan et al., 2002a; Newman-Tancredi et al., 2003) and human brain membranes (Elliott and Reynolds, 1999; Gonzalez-Maeso et al., 2000), and at 5-HT_{1A} receptors heterologously expressed in cell membranes (Bertin et al., 1992; Newman-Tancredi et al., 1996). The present study investigated the in vitro functional profile of OPC-14523 at 5-HT_{1A} receptors in rat brain tissue sections and membranes, in an attempt to better interpret the antidepressant-like effects of OPC-14523 in rodents, and at native and recombinant human 5-HT_{1A} receptors, to support the continued development of OPC-14523 as an antidepressant primarily targeting 5-HT_{1A} receptors. In vitro autoradiography was used to quantitatively assess the effects of OPC-14523 on regional [^{35}S]GTPγS binding to horizontal rat brain sections. OPC-14523 was also studied to estimate its potency and relative intrinsic activity in [^{35}S]GTPγS binding assays using membranes prepared from rat hippocampus, postmortem human frontal cortex and Chinese Hamster Ovary (CHO) cells stably expressing cloned human 5-HT_{1A} (h5-HT_{1A}) receptors. The term relative intrinsic activity is used herein to refer to the maximal response generated by each compound relative to that produced by a reference 5-HT_{1A} full agonist in the same experiment, in the current studies this reference compound is 5-HT or (+)8-OH-DPAT. These membrane-based [^{35}S]GTPγS binding assays were also used to profile the activity of (−)pindolol at 5-HT_{1A} receptors, which, to our knowledge, has not been studied to date at postmortem human 5-HT_{1A} receptors. Brain tissues and membranes were selected for use in the majority of these studies, as they enable estimates of drug potency and relative intrinsic activity to be acquired from 5-HT_{1A} receptors natively expressed in the presence of their cognate G-proteins and associated signal transduction biochemistry. In vitro radioligand saturation and competition binding assays were also performed to estimate, for the first time, the binding affinity characteristics of hydrogen-3 8-hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) and OPC-14523 at 5-HT_{1A} receptors expressed in membranes prepared from postmortem human frontal cortex.

2. Methods

2.1. Materials

[^{35}S]GTPγS (1200 Ci/mmol), [^{3}H]8-OH-DPAT (170 Ci/mmol) and CHO-h5-HT_{1A} cell membranes (density of receptor expression (B_{max})=1.0 pmol/mg membrane protein) were purchased from NEN Life Science Products (Boston, MA). Frontal cortex tissue was obtained from Dr. Ronald Hamilton (University of Pittsburgh Medical Center, PA), which had been dissected and snap-frozen at 6 h postmortem from a 59-year-old male with no previous history of neurological or psychiatric disease. OPC-14523 was synthesized by Otsuka Pharmaceutical Co. Ltd. (Tokushima, Japan). 5-HT, (+)8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin, (−)8-OH-DPAT, buspirone, (−)pindolol, 1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine (NAN-190), spiperone, WAY-100635, GDP, and all other assay reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO).

2.2. Animals

Male Sprague–Dawley rats (Charles River Laboratories, Raleigh, NC) were sacrificed by decapitation at a weight of 270 to 330 g, and their brains were removed and either immediately frozen in isopentane and stored at −20 °C for use in the autoradiography [^{35}S]GTPγS binding assay, or homogenized to prepare hippocampal membranes. Experimental protocols for all animal studies were approved and conducted in accordance with Otsuka Maryland Medicinal Laboratories' Animal Care and Use Committee (IACUC).

2.3. Autoradiographic [^{35}S]GTPγS binding assay

Frozen rat brains were cut on a cryostat at −17 °C into 20 μm thick horizontal sections, thaw mounted onto gelatin coated slides, and stored at − 135 °C. (+)8-OH-DPAT and OPC-14523 were each studied at a 1 μM concentration for their effects on [^{35}S]GTPγS binding to horizontal rat brain sections in the presence and absence of WAY-100635. Sections were equilibrated in assay buffer (50 mM Tris–HCl, 3 mM $MgCl_2$, 0.2 mM EGTA, 100 mM NaCl, pH=7.4) for 10 min, and then incubated for 20 min in assay buffer

containing 2 mM GDP. Sections were subsequently incubated for 2 h in assay buffer containing 2 mM GDP, 50 pM [^{35}S]GTPγS and vehicle or test drug, after which they were rinsed twice with ice-cold assay buffer and once with ice-cold water, prior to being air-dried and exposed to Kodak Biomax MR film for 15 h. Autoradiographs were digitized by light transmission scanning and densitometric image analysis was performed using Scion Image software (Scion Corporation, Frederick, MD).

2.4. Preparation of rat hippocampal and human frontocortical membranes

Brain tissues were homogenized using a Polytron (Brinkman) in 10 volumes of ice-cold rat hippocampal tissue buffer (50 mM Tris–HCl, 1 mM dithiothreitol and 1 mm EGTA, pH=7.4) or human frontal cortex tissue buffer (20 mM HEPES, 10 mM EDTA and Sigma protease inhibitor cocktail, pH=7.4). The homogenate was centrifuged (1000 ×g for 5 min at 4 °C) and the resultant supernatant (S1) removed and stored on ice. The remaining pellet was resuspended in buffer and homogenized and centrifuged as before. The supernatant was then mixed with S1, centrifuged (11 000 ×g for 20 min at 4 °C), and the final membrane pellet was resuspended in buffer (protein concentration ~4 mg/ml determined by the Bradford method; Sigma-RBI) and stored at − 80 °C.

2.5. Drug effects on [^{35}S]GTPγS binding to rat hippocampal, human frontocortical and CHO-h5-HT$_{1A}$ cell membranes

Estimates of potency (pEC$_{50}$) and relative intrinsic activity (E_{max}, maximal drug effect on [^{35}S]GTPγS binding expressed as a percentage of the effect of 10 μM (+)8-OH-DPAT (rat and human membrane assays) or 10 μM 5-HT (CHO-h5-HT$_{1A}$ cell membrane assay)) were obtained for 5-HT, (+)8-OH-DPAT, (−)8-OH-DPAT, buspirone, OPC-14523, (−)pindolol, NAN-190, spiperone and WAY-100635. All drugs were tested in triplicate at 8 to 10 different concentrations. For the rat and human membrane assays, drugs were preincubated for 30 min at 30 °C in buffer (50 mM Tris–HCl, 100 mM NaCl, 1 mM $MgCl_2$, 1 mM EGTA, 1 mM dithiothreitol, pH=7.4) mixed with membranes (20 μg protein) and GDP (100 and 10 μM for rat and human membrane assays, respectively), prior to being mixed with [^{35}S]GTPγS (0.2 nM) and incubated further for 30 min. The CHO-h5-HT$_{1A}$ cell membrane assays were performed by incubating drugs for 60 min at 22 °C in buffer (25 mM Tris–HCl, 50 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EGTA, pH=7.4) mixed with membranes (10 μg protein; NEN Life Science Products, Boston, MA), GDP (1 μM) and [^{35}S]GTPγS (0.1 nM). All assays were terminated by rapid filtration through Whatman GF/B filter paper using a Brandel harvester, and radioactivity bound to the filter paper was measured by liquid scintillation counting (Clinigamma, LKB/Wallach). The same assay conditions were also used to determine the inhibitory potency (pIC$_{50}$) of WAY-100635, tested at concentrations ranging 0.01 nM to 50 μM, against 1 μM (rat hippocampal membrane assay) and 100 nM (human frontal cortex membrane assay) concentrations each of 5-HT, (+)8-OH-DPAT and OPC-14523, and against 1 μM concentrations each of 5-HT and OPC-14523 (CHO-h5-HT$_{1A}$ cell membrane assay).

2.6. Radioligand binding assays

The 5-HT$_{1A}$ receptor radioligand binding assays were performed by incubating human frontocortical membranes (15–20 μg protein) with [^{3}H]8-OH-DPAT in buffer (50 mM Tris–HCl, 10 mM $MgSO_4$, 0.5 mM EDTA and 0.1% ascorbic acid, pH=7.4) at room temperature for 60 min. All assays were terminated by rapid filtration through Whatman GF/B filter paper presoaked and then washed in 50 mM Tris–HCl, pH=7.4. Bound radioactivity was determined by beta counting and non-specific binding was defined in the presence of 10 μM (+)8-OH-DPAT. Saturation binding assays were carried out by incubating membranes with 8 different concentrations of [^{3}H]8-OH-DPAT (0.05 to 2.5 nM) and specific binding data was analyzed using non-linear regression to provide estimates of binding affinity (K_d) and density of receptor expression (B_{max}). Competition binding assays were also performed by incubating frontocortical membranes with 1 nM [^{3}H]8-OH-DPAT and 9 different concentrations each of OPC-14523, 5-HT, buspirone and WAY-100635, and the binding affinity (K_i) of OPC-14523, 5-HT, buspirone and WAY-100635 was in each case calculated by the equation, $K_i=(IC_{50})/(1+([radioligand]/K_d)$ using non-linear regression analysis.

2.7. Data analysis

All estimates of receptor binding affinity, density of receptor expression, potency, relative intrinsic activity and inhibitory potency were determined by non-linear regression analysis of each binding isotherm using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, CA). The same software application was used to perform an F test on each set of [^{3}H]8-OH-DPAT radioligand binding data, to determine whether they fitted more closely to equations describing one or two site receptor binding models. Data were analyzed using the results from three representative experiments.

3. Results

3.1. Autoradiographic [^{35}S]GTPγS binding assay

OPC-14523 and (+)8-OH-DPAT both produced increases in [^{35}S]GTPγS binding to rat brain sections that, as shown in Fig. 1, were most prominent in the hippocampus, septum, frontal cortex and entorhinal cortex. Densitometric analysis of a region of interest drawn in the hippocampus of these autoradiographs revealed that OPC-14523 increased [^{35}S]GTPγS binding in rat hippocampus to a level approximately half (53%) of that produced by an equivalent concentration of (+)8-OH-DPAT. These effects of 1 μM concentrations of OPC-14523 and (+)8-OH-DPAT on [^{35}S]GTPγS binding were not seen in the presence of a 20 μM concentration of WAY-100635 (Fig. 1).

3.2. [^{35}S]GTPγS binding to rat hippocampal membranes

Table 1 and Fig. 2 show the effects of OPC-14523 and reference drugs on [^{35}S]GTPγS binding to rat hippocampal membranes. OPC-14523 (pEC$_{50}$=7.60±0.23) and (+)8-OH-DPAT (pEC$_{50}$=7.60±0.13) produced similar and more potent increases in [^{35}S]GTPγS binding than 5-HT (pEC$_{50}$=6.64±0.09) and all other reference drugs tested. OPC-14523 behaved as a partial agonist (E_{max}=41.1% relative to the effect of 10 μM (+)8-OH-DPAT on [^{35}S]GTPγS binding) as did (−)8-OH-DPAT and buspirone, whereas (+)8-OH-DPAT behaved as a full agonist.

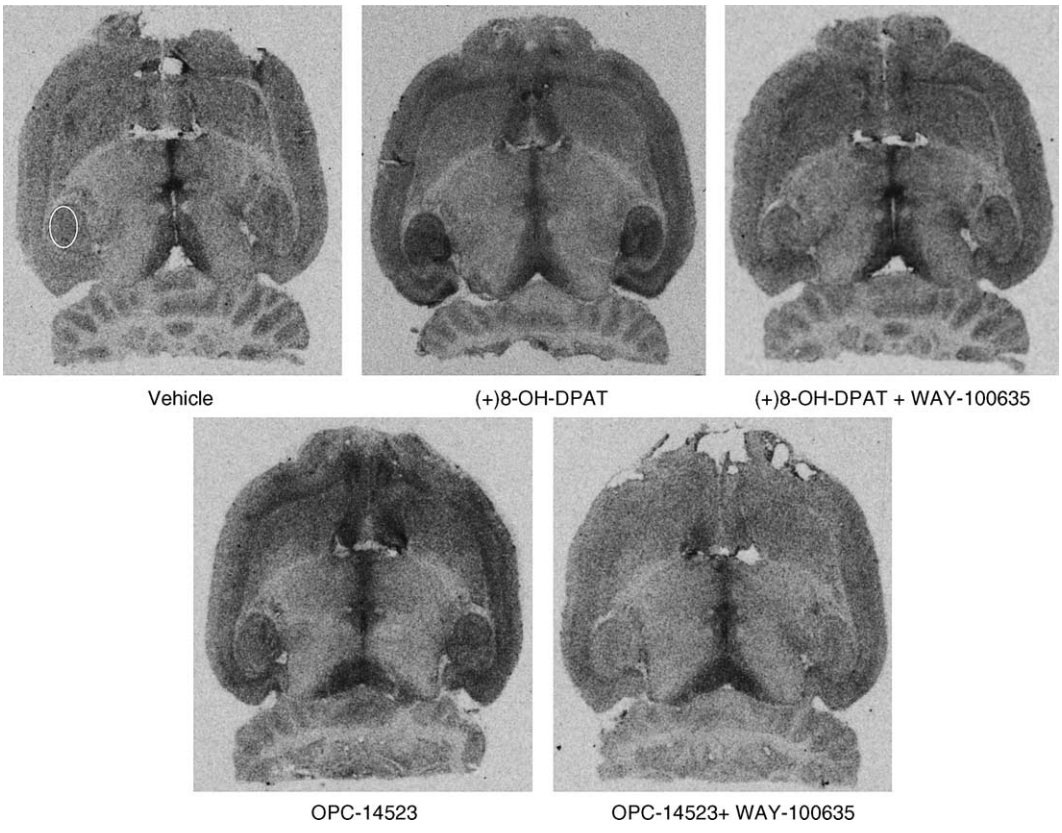

Fig. 1. Autoradiographic images of the effects of vehicle, (+)8-OH-DPAT (1 μM), (+)8-OH-DPAT (1 μM)+WAY-100635 (20 μM), OPC-14523 (1 μM) and OPC-14523+WAY-100635 (20 μM) on [^{35}S]GTPγS binding to horizontal rat brain sections. A white ellipse is shown on the top left autoradiograph to define the region of interest used for densitometric image analysis.

5-HT increased [^{35}S]GTPγS binding to rat hippocampal membranes to a greater extent than all other compounds tested (E_{max}=125.9%). In contrast, (−)pindolol, NAN-190, spiperone and WAY-100635 all failed to produce changes in [^{35}S]GTPγS binding. WAY-100635 potently blocked all of the effects of 1 μM concentrations of OPC-14523 and (+)8-OH-DPAT on [^{35}S]GTPγS

Table 1
Functional parameter estimates for OPC-14523 and reference drugs in [^{35}S]GTPγS binding assays using membranes prepared from rat hippocampus, human frontal cortex, and CHO cells stably expressing cloned human 5-HT$_{1A}$ receptors (CHO-h5-HT$_{1A}$ cells)

Agonist	Rat Hippocampus			Human Frontal Cortex			CHO-h5-HT$_{1A}$ Cells		
	pEC$_{50}$±SEM	E_{max}±SEM (%)	R^2	pEC$_{50}$±SEM	E_{max}±SEM (%)	R^2	pEC$_{50}$±SEM	E_{max}±SEM (%)	R^2
5-HT	6.64±0.09	125.9±4.4	0.99	7.16±0.13	125.2±6.3	0.98	8.34±0.08	102.7±2.7	0.99
(+)8-OH-DPAT	7.10±0.07	102.3±2.4	0.99	7.45±0.10	92.5±3.4	0.99	8.29±0.07	102.7±2.2	0.99
(−)8-OH-DPAT	7.60±0.13	49.6±1.8	0.98	7.50±0.20	51.2±3.2	0.95	8.19±0.07	85.4±2.0	0.99
Buspirone	6.87±0.15	29.8±1.9	0.97	7.04±0.11	48.3±2.2	0.98	7.64±0.09	85.2±2.5	0.99
OPC-14523	7.60±0.23	41.1±2.9	0.92	7.89±0.08	64.0±1.8	0.99	8.00±0.11	85.5±2.6	0.98
(−)Pindolol	Inactive			6.23±0.47	39.8±12.0	0.74	8.01±0.26	30.8±2.0	0.86
NAN-190	Inactive			6.53±0.26	40.1±5.1	0.92	8.71±0.30	28.1±1.7	0.87
Spiperone	Inactive			Inactive			7.75±0.08	− 22.0±1.0	0.99
WAY-100635	Inactive			Inactive			Inactive		
WAY-100635 inhibition	pIC$_{50}$±SEM		R^2	pIC$_{50}$±SEM		R^2	pIC$_{50}$±SEM		R^2
5-HT	9.07±0.16		0.99	9.49±0.12		0.99	6.88±0.12		0.98
(+)8-OH-DPAT	9.04±0.14		0.98	9.28±0.14		0.99	Not tested		
OPC-14523	8.69±0.25		0.95	8.76±0.27		0.96	6.56±0.20		0.95

Agonist potency (pEC$_{50}$) and relative intrinsic activity (E_{max}, maximal drug effect upon basal [^{35}S]GTPγS binding expressed as a percentage of that produced by 10 μM (+)8-OH-DPAT or, in the CHO-h5-HT$_{1A}$ assay, 10 μM 5-HT) were estimated by non-linear regression analysis of the data shown in Figs. 2–4. Non-linear regression was also used to estimate the inhibitory potency (pIC$_{50}$) of WAY-100635, tested at concentrations ranging 0.01 nM to 50 μM, against 1 μM (rat hippocampus and CHO-h5-HT$_{1A}$ cell membrane assays) and 100 nM (human frontal cortex assay) concentrations each of OPC-14523, 5-HT and (+)8-OH-DPAT. R^2 represents the goodness of fit between observed concentration effect data points and non-linear functions derived for each drug or drug combination studied. All data points are means±SEM of three representative experiments each containing triplicate reactions.

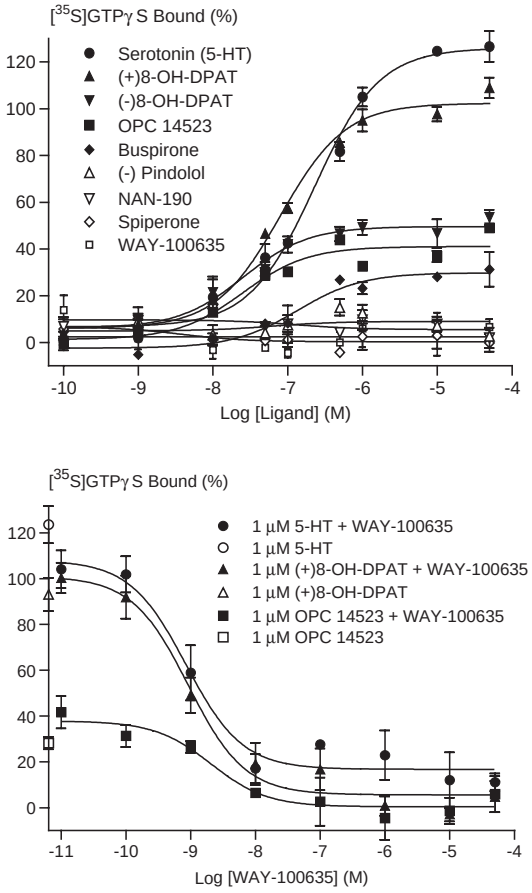

Fig. 2. Drug effects on [^{35}S]GTPγS binding to rat hippocampal membranes. All data points are means ± SEM of three representative experiments containing triplicate reactions, and are expressed as a percentage of the stimulatory effect of 10 μM (+)8-OH-DPAT on [^{35}S]GTPγS binding.

binding. WAY-100635 also potently inhibited, but did not fully abolish, 5-HT-induced increases in [^{35}S]GTPγS binding to rat hippocampal membranes.

3.3. [^{35}S]GTPγS binding to human frontocortical membranes

The effects of OPC-14523 and reference drugs on [^{35}S]GTPγS binding to human frontocortical membranes are shown in Table 1 and Fig. 3. OPC-14523 produced more potent increases in [^{35}S]GTPγS binding to human frontocortical membranes (pEC_{50} = 7.89 ± 0.08) than all other compounds tested. OPC-14523 behaved as a partial agonist with a relative intrinsic activity (E_{max} = 64% relative to the effect of 10 μM (+)8-OH-DPAT on [^{35}S]GTPγS binding) exceeding that produced by (−)8-OH-DPAT, buspirone, (−)pindolol and NAN-190. In comparison, 5-HT produced greater increases in [^{35}S]GTPγS binding than all other compounds tested (E_{max} = 125.2%). In contrast, WAY-100635 and spiperone both failed to stimulate [^{35}S]GTPγS binding on their own. Nevertheless, WAY-100635 potently blocked, in a concentration-dependent manner in each case, all of the stimulatory effects of 100 nM concentrations of OPC-14523 and (+)8-OH-DPAT upon [^{35}S]GTPγS binding. WAY-100635 was also a potent inhibitor of 5-HT activity in this assay, although WAY-100635 was unable to fully block 5-HT-induced [^{35}S]GTPγS binding.

3.4. [^{35}S]GTPγS binding to CHO-h5-HT$_{1A}$ cell membranes

OPC-14523 and reference drug effects on [^{35}S]GTPγS binding to CHO-h5-HT_{1A} receptors are shown in Table 1 and Fig. 4. OPC-14523 (pEC_{50} = 8.0 ± 0.11), (−)8-OH-DPAT, (−)pindolol and NAN-190 all behaved as similarly potent, partial agonists, albeit with varying relative intrinsic activities, and buspirone was comparatively less potent in this respect. Nevertheless, buspirone exhibited a relative intrinsic activity almost identical to that of OPC-14523 (E_{max} = 85.5% relative to the effect of 10 μM 5-HT on [^{35}S]GTPγS binding) and (−)8-OH-DPAT, and these relative intrinsic activity values were several-fold higher than those of (−)pindolol and NAN-190. In comparison, 5-HT and (+)8-OH-DPAT were almost identically potent, full agonists in their respective stimulation of [^{35}S]GTPγS binding to CHO-h5-HT_{1A} receptors. In contrast, spiperone exhibited a moderately potent, inverse agonist activity (pEC_{50} = 7.75 ± 0.08; E_{max} = −22.0%), while WAY-100635, which was inactive on its own, concentration-dependently blocked all of the effects of 1 μM concentrations each of 5-HT and OPC-14523 on [^{35}S]GTPγS binding.

3.5. Radioligand binding assays

[^{3}H]8-OH-DPAT displayed specific saturable binding to human frontocortical membranes. The specific binding of this radioligand

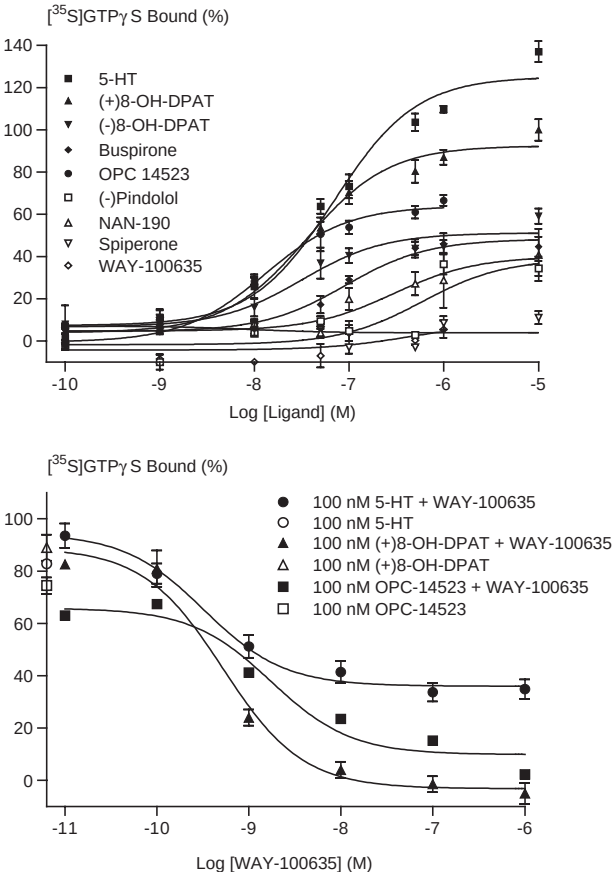

Fig. 3. Drug effects on [^{35}S]GTPγS binding to membranes prepared from human frontal cortex. All data points are means ± SEM of three representative experiments containing triplicate reactions, and are expressed as a percentage of the stimulatory effect of 10 μM (+)8-OH-DPAT on [^{35}S]GTPγS binding.

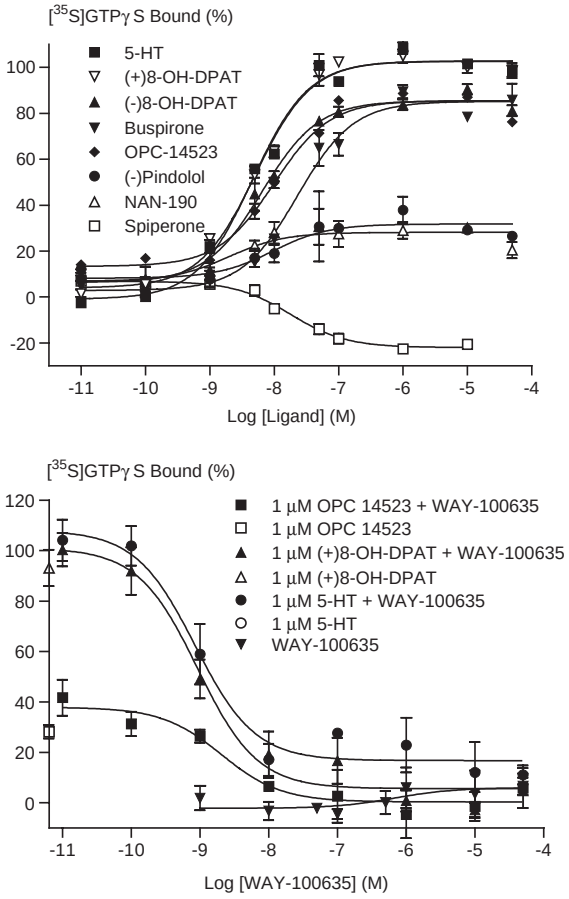

Fig. 4. Drug effects on [^{35}S]GTPγS binding to CHO-h5-HT$_{1A}$ cell membranes. All data points are means±SEM. of three representative experiments containing triplicate reactions, and are expressed as a percentage of the stimulatory effect of 10 μM 5-HT on [^{35}S]GTPγS binding.

represented more than 70% of total binding, whereas non-specific binding increased linearly with increasing concentrations of [^{3}H]8-OH-DPAT. The range of DPM values in a representative saturation binding assay were as follows: 0.05 nM [^{3}H](+)8-OH-DPAT total bound (116 to 120 DPM) and non-specific (31–35 DPM); 2.5 nM [^{3}H](+)8-OH-DPAT total bound (799 to 874 DPM) and non-specific (455–510 DPM). Analysis of these saturation binding data showed that [^{3}H]8-OH-DPAT bound to a single site ($P<0.05$, *F* Test) on human frontocortical membranes ($K_d=0.17\pm0.03$ nM), which was expressed at a density (B_{max}) of 34.64±1.44 fmol/mg protein. Analysis of our competition binding assay data showed that OPC-14523 ($pK_i=8.93\pm0.09$, $pIC_{50}=8.10\pm0.25$), 5-HT ($pK_i=9.01\pm0.13$, $pIC_{50}=8.62\pm0.04$), buspirone ($pK_i=8.42\pm0.17$, 0.17, $pIC_{50}=8.03\pm0.06$) and WAY-100635 ($pK_i=9.96\pm0.01$, $pIC_{50}=9.60\pm0.05$) each bound with high affinity and displaced [^{3}H](+)8-OH-DPAT to a similar extent (~85–88%) to that of 10 μM (+)8-OH-DPAT from a single site ($P<0.05$ in each case, *F* Test) on human frontocortical membranes.

4. Discussion

The present study used [^{35}S]GTPγS binding assays to characterize the in vitro functional profile of the antidepressant drug candidate OPC-14523 at rat and human 5-HT$_{1A}$ receptors. Radioligand binding assays were also performed to estimate the binding affinity of OPC-14523 at 5-HT$_{1A}$ receptors in human frontocortical membranes. OPC-14523 increased [^{35}S]GTPγS binding most prominently in the hippocampus, septum, frontal cortex and entorhinal cortex of rat brain tissue sections. These effects of OPC-14523 were mediated by 5-HT$_{1A}$ receptors, as they occurred preferentially in brain regions densely populated with 5-HT$_{1A}$ receptors and were blocked by WAY-100635. Furthermore, the selective 5-HT$_{1A}$ receptor full agonist (+)8-OH-DPAT increased [^{35}S]GTPγS binding to an even greater extent in the same brain regions, and these regionally specific effects of (+)8-OH-DPAT were also abolished by WAY-100635. OPC-14523 also behaved as a potent, partial agonist in its stimulation of [^{35}S]GTPγS binding to rat hippocampal membranes, and these effects of OPC-14523 were blocked by WAY-100635. Of interest was the finding that OPC-14523 displayed a relative intrinsic activity in rat hippocampal membranes comparable to that detected in rat brain sections. These in vitro demonstrations that OPC-14523 is a potent, partial agonist at rat brain 5-HT$_{1A}$ receptors are in agreement with in vivo behavioral evidence that OPC-14523 activates 5-HT$_{1A}$ receptors (Oshiro et al., 2000; Tottori et al., 2001). Likewise, OPC-14523 displayed a potent, partial agonist profile in its stimulation of [^{35}S]GTPγS binding to membranes prepared from both human frontal cortex and CHO-h5-HT$_{1A}$ cells, and these data are in accordance with our demonstration that OPC-14523 binds with high affinity to human frontocortical 5-HT$_{1A}$ receptors.

The present classification of OPC-14523 as a 5-HT$_{1A}$ receptor partial agonist in rat brain suggests the antidepressant-like responses of this compound might be mediated through its combined activation of presynaptic somatodendritic 5-HT$_{1A}$ receptors, which are more hypersensitive due to them having a receptor reserve, and functional inhibition of postsynaptic 5-HT$_{1A}$ receptors, which have little or no receptor reserve (Meller et al., 1990; Yocca et al., 1992). However, the antidepressant-like activity of OPC-14523 in rats is also blocked by the σ receptor antagonist NE-100, suggesting that OPC-14523 might direct 5-HT$_{1A}$ receptors to transduce signaling through a biochemical pathway regulated by σ receptor function. Studies are ongoing in our laboratory to profile the in vitro functional characteristics of OPC-14523 at σ receptors stably expressed in CHO cells, although repeated attempts to identify a functional response to a variety of σ receptor agonists have all been without success.

(−)Pindolol and NAN-190 both displayed partial agonist activities at h5-HT$_{1A}$ receptors whilst failing to activate rat hippocampal 5-HT$_{1A}$ receptors. These data are consistent with the effects of these drugs on [^{35}S]GTPγS binding to h5-HT$_{1A}$ receptors (Newman-Tancredi et al., 1998; Stanton and Beer, 1997) and rat brain tissue sections (Newman-Tancredi et al., 2001; Waeber and Moskowitz, 1997). To our

knowledge, we are the first to demonstrate a 5-HT_{1A} receptor partial agonist profile for (−)pindolol at postmortem human frontocortical 5-HT_{1A} receptors. This effect of (−)pindolol would not be expected to result from an interaction with other subtypes of frontocortical 5-HT_1 receptors that also elicit [^{35}S]GTPγS binding, as (−)pindo-)pindolol binds with a moderate affinity to rat 5-HT_{1B} receptors (Millan et al., 2002), but with low micromolar affinity to human 5-HT_{1B} and 5-HT_{1D} receptors (Weinshank et al., 1992). (−)Pindolol is believed to accelerate the antidepressant activity of SSRIs through its blockade of somatodendritic 5-HT_{1A} autoreceptors (Artigas et al., 1996; Blier and Bergeron, 1996). Acting as a 5-HT_{1A} receptor partial agonist, (−)pindolol might improve SSRI antidepressant therapy by acting as a combined functional agonist and antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors, respectively, as discussed above for OPC-14523. (−)Pindolol would also be predicted to exert agonist and antagonist effects at 5-HT_{1A} receptors that have low and high levels of tonic 5-HT activity, respectively.

(+)8-OH-DPAT was a potent, full agonist at rat hippocampal, cloned human and human frontocortical 5-HT_{1A} receptors, and, in each case, these effects of (+)8-OH-DPAT were concentration-dependently abolished by WAY-100635, confirming that the current [^{35}S]GTPγS binding assays all provided reliable estimates of drug activity at 5-HT_{1A} receptors. On the other hand, the endogenous 5-HT_{1A} receptor full agonist 5-HT effected greater increases in [^{35}S]GTPγS binding to rat hippocampal and human frontocortical membranes than (+)8-OH-DPAT and all other compounds tested. These enhanced effects of 5-HT most likely represent combined effects of 5-HT at 5-HT_{1A}, and 5-HT_{1B} or 5-HT_{1D} receptors, all of which are located in rat hippocampus and human frontal cortex, and when activated can increase [^{35}S]GTPγS binding (_Hlt105037560[Waeber and Moskowitz, 1997; Dupuis et al., 1999; Herrick-Davis et al., 1988).

The present in vitro functional characteristics of (+)8-OH-DPAT, (−)8-OH-DPAT, buspirone, spiperone and WAY-100635 at rat hippocampal and h5-HT_{1A} receptors are similar to previously published estimates for these drugs (Alper and Nelson, 1998; Jordan et al., 2002a,b; Newman-Tancredi et al., 2003; Pauwels et al., 1997; Stanton and Beer, 1997; Lejeune et al., 1997; Newman-Tancredi et al., 1996). Our present estimates of drug potency at rat hippocampal and human frontal cortical 5-HT_{1A} receptors are generally weaker than corresponding potency estimates generated for the same drugs tested at h5-HT_{1A} receptors in this and a previous study (Jordan et al., 2002b). This difference may be related to many different biochemical and physiological factors. For example, the concentration of GDP used in the rat and human brain assays was respectively 10 and 100-fold higher than that used in the CHO-h5-HT_{1A} assay. Estimates of binding affinity and potency at 5-HT_{1A} receptors are dramatically affected by increasing concentrations of GDP (Schlegel and Peroutka, 1986; Alper and Nelson, 1998; Gonzalez-Maeso et al., 2000). Nevertheless, higher concentrations of GDP are typically required to achieve optimal assay sensitivity in [^{35}S]GTPγS binding assays using membranes prepared from brain tissues versus recombinant cells. A higher GDP concentration may be required due to differences in basal or constitutive activity between brain and cell membranes, which in turn would affect basal [^{35}S]GTPγS binding and assay sensitivity. Regardless, OPC-14523 was slightly more potent than the prototypical 5-HT_{1A} receptor agonist (+)8-OH-DPAT at rat and human frontocortical 5-HT_{1A} receptors, studied under the same experimental conditions in each case.

Estimates of relative intrinsic activity for (−)8-OH-DPAT, buspirone and OPC-14523, generated using rat and human brain assays, were each lower than their respective values detected using CHO-h5-HT_{1A} receptors. Such differences may be related to the existence of a large receptor reserve in the CHO-h5-HT_{1A} receptor membranes, compared with the rat and human brain membranes. Indeed, in the current study, 5-HT_{1A} receptors were expressed at an almost 30-fold higher level in CHO-h5-HT_{1A} cell membranes than in human frontocortical membranes. Inverse relationships have been demonstrated between receptor density and estimated values of relative intrinsic activity (Adham et al., 1993; Burris et al., 2002), although this does not explain why NAN-190 exhibited a substantially higher relative intrinsic activity at native versus h5-HT_{1A} receptors. Estimates of relative intrinsic activity also appear to be dependent on whether the receptor being studied is constitutively active in a particular test system, which might contribute to the inactivity of spiperone at rat hippocampal and human frontocortical 5-HT_{1A} receptors, compared with its inverse agonist at constitutively active CHO-h5-HT_{1A} receptors in this and previous studies (Newman-Tancredi et al., 1997; Jerning et al., 2002). In vitro functional activity also appears to be dependent on the availability of specific biochemical intracellular signal transduction elements, such as specific G protein subunits and accessory binding proteins, which may be differentially expressed in brain and recombinant cell membranes (Kenakin, 2001, 2002), as well as the phenomenon of agonist directed trafficking, a concept largely developed by Kenakin (1995a,b), whereby agonists may differ in their ability to direct a single G protein-coupled receptor to activate one or more of its associated signal transduction pathways. Cell specific G protein expression and agonist directed trafficking might also contribute to the higher relative intrinsic activity estimates displayed by some drugs at cloned versus native human 5-HT_{1A} receptors, as well as the contrasting in vitro functional profiles of WAY-100635, which has demonstrated silent antagonist and inverse agonist activity at h5-HT_{1A} receptors expressed in CHO (Newman-Tancredi et al., 1996) and human epithelioid carcinoma (HeLa) cells (Cosi and Koek, 2000), respectively. Regional differences in G protein subunit expression should be considered when interpreting drug activity in brain sections using autoradiographic [^{35}S]GTPγS binding assays. Such differences may also

account for variations in basal [^{35}S]GTPγS binding in different rat brain regions, which, in the case of the hypothalamus and amygdala, can be high enough to prevent reliable quantitation of agonist-stimulated [^{35}S]GTPγS binding (see Hensler, 2003).

In summary, OPC-14523 specifically increased [^{35}S]GTPγS binding in areas of rat brain densely populated with 5-HT$_{1A}$ receptors, where it behaved as a partial agonist relative to (+)8-OH-DPAT, and displayed potent, partial agonist activities at rat hippocampal, human frontocortical and CHO-h5-HT$_{1A}$ receptors. These data support the continued development of OPC-14523 as a novel antidepressant candidate whose mechanism of action involves potent partial agonist activity at 5-HT$_{1A}$ receptors.

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