Neuropsychopharmacology (2004) 29, 1688–1698

www.neuropsychopharmacology.org

Preferential 5-HT$_{1A}$ Autoreceptor Occupancy by Pindolol is Attenuated in Depressed Patients: Effect of Treatment or an Endophenotype of Depression?

Eugenii A Rabiner*[,1,2], Zubin Bhagwagar[2,3], Roger N Gunn[1,4], Philip J Cowen[3] and Paul M Grasby[2]

[1]PET Psychiatry, Translational Medicine and Technologies, GlaxoSmithKline, Cambridge, UK; [2]MRC Cyclotron Unit, Hammersmith Hospital, Imperial College School of Medicine, London, UK; [3]Department of Psychiatry, Warneford Hospital, University of Oxford, Oxford, UK; [4]McConnell Brain Imaging Center, Montreal Neurological Institute, Montreal, Canada

Using positron emission tomography and the selective 5-HT$_{1A}$ receptor radioligand [^{11}C]WAY100635, we previously demonstrated a preferential occupancy of 5-HT$_{1A}$ autoreceptors, compared to postsynaptic receptors by pindolol in healthy volunteers. We have speculated that preferential occupancy may be clinically important for the purported actions of pindolol in accelerating the antidepressant effects of selective serotonin re-uptake inhibitors (SSRIs). In this study, we have examined the preferential occupancy by pindolol of 5-HT$_{1A}$ autoreceptors, following three different pindolol regimes (10 mg single dose, 2.5 mg t.i.d., and 5 mg t.i.d., in 15 depressed patients on SSRIs. In addition, seven healthy volunteers were examined following a single 10 mg dose of pindolol. We found a preferential occupancy of 22.6 $\pm$ 7.7% following a single dose of 10 mg of pindolol, in the healthy volunteers, which was attenuated in depressed patients on the same dose of pindolol to 2.9 $\pm$ 10.8% (Student's $t = 3.94$, df = 12, $p = 0.002$). In addition, we found a significant negative correlation between the degree of preferential occupancy and the severity of depression as assessed by the Hamilton depression rating score (HAM-D), Spearman's $\rho = -0.728$, $N = 14$, $p = 0.003$, in the depressed sample. A possible mechanism underlying preferential occupancy and the attenuation of this phenomenon in depressed patients on SSRIs may include changes in the proportion of high affinity 5-HT$_{1A}$ sites in the autoreceptor region of the midbrain raphe. Speculatively, the degree of preferential occupancy may serve as a surrogate marker for depression, or the pharmacological effects of antidepressants.
Neuropsychopharmacology (2004) **29,** 1688–1698, advance online publication, 5 May 2004; doi:10.1038/sj.npp.1300472

Keywords: receptors; serotonin; neurotransmitter; antidepressive agents; pindolol/administration and dosage; brain/drug effects; human

INTRODUCTION

Positron emission tomography (PET) studies examining pindolol occupancy of the 5-HT$_{1A}$ receptor in healthy volunteers *in vivo* have found a higher occupancy at the autoreceptor as compared to the postsynaptic receptor (Martinez *et al*, 2001; Rabiner *et al*, 2000). Similarly, a preferential occupancy at the autoreceptor has been reported in a PET study in the rat (Hirani *et al*, 1999), as well as in some (Castro *et al*, 2000; Serrats *et al*, 2000), but not all (Raurich *et al*, 1999), *in vitro* human and rat studies. The significance of this finding is not clear, as the available evidence indicates that the 5-HT$_{1A}$ autoreceptor and postsynaptic receptor are structurally identical (Albert *et al*, 1990; Radja *et al*, 1992). On the other hand, considerable evidence exists for differences between the autoreceptors and postsynaptic receptors *in vivo*, such as differences in receptor reserve between the two sites (Meller *et al*, 1990).

An earlier study from our unit (Rabiner *et al*, 2001) reported the 5-HT$_{1A}$ autoreceptor occupancy by pindolol in depressed patients on selective serotonin re-uptake inhibitors (SSRIs). An incidental finding of that study, not reported in our original publication, but presented here, was the attenuation of the expected preferential occupancy of the 5-HT$_{1A}$ autoreceptor in this group. Activation of the 5-HT$_{1A}$ autoreceptors inhibits 5-HT neurone firing, and hence 5-HT release in terminal synapses (Adell and Artigas, 1991; Invernizzi *et al*, 1992; Sharp *et al*, 1989; VanderMaelen *et al*, 1986). Activation of the postsynaptic 5-HT$_{1A}$ receptors mediates some of the effects of 5-HT released from the nerve terminals (Blier *et al*, 1987; Chaput *et al*, 1991; Invernizzi *et al*, 1991; Sinton and Fallon, 1988), and may be critical to the antidepressant action of SSRIs. As preferential

*Correspondence: Dr EA Rabiner, PET Psychiatry, Tanslational Medicine and Technologies, GlaxoSmithKline, Addenbrooke's Hospital, Cambridge CB2 2GG, UK, Tel: +44 1223 296072, Fax: +44 1223 296108, E-mail: Eugenii_A_Rabiner@gsk.com
Received 29 August 2003; revised 08 March 2004; accepted 12 March 2004
Online publication: 29 March 2004 at http://www.acnp.org/citations/Npp03290403397/default.pdf

binding at the autoreceptor may be crucial for the proposed mechanism of action of pindolol in augmenting SSRIs (Artigas *et al*, 2001; Martinez *et al*, 2001; Rabiner *et al*, 2000), we further investigated pindolol 5-HT$_{1A}$ occupancy effects in a larger group of controls and depressed patients.

In this study we used [^{11}C]WAY-100635 PET to examine a further group of seven depressed patients on SSRIs treatment before and after a single oral dose of 10 mg of pindolol, a dose known to produce consistent preferential occupancy in healthy volunteers (Rabiner *et al*, 2000). This group of seven depressed patients was compared to a group of seven healthy volunteers, who were also examined with [^{11}C]WAY-100635 PET before and after a single oral dose of 10 mg of pindolol.

MATERIALS AND METHODS

Subjects

All studies were approved by the Imperial College School of Medicine Ethics Committee and the Administration of Radioactive Substances Advisory Committee. All subjects underwent a psychiatric interview with a qualified psychiatrist and a full physical examination, and gave written informed consent to the study. Depressed patients were treated with a variety of SSRIs (Paroxetine 20–40 mg, Fluoxetine 20–40 mg, Sertraline 50–100 mg), or Venlafaxine (75 or 150 mg). The 17-item Hamilton Depression Rating Scale and the 21-item Beck's Depression Questionaire were collected from all depressed subjects at the time of the pindolol scan.

Study 1

The methodology of this study has been described in our previous publication (Rabiner *et al*, 2001). Briefly, eight patients (seven M, one F, ages 24–61) meeting DSM-IV criteria for MDD, on therapeutic doses of antidepressant medication only (SSRI $n=7$, Venlafaxine $n=1$), and not fully recovered were examined. All subjects received two open label [^{11}C]WAY-100635 PET scans. The first was a baseline scan (on antidepressant treatment only), while the second was conducted following a 7–14 days period of augmentation with pindolol 2.5 mg t.i.d. ($n=4$), or 5 mg t.i.d. ($n=4$). Although the autoreceptor occupancy by pindolol in this group was reported previously, preferential occupancy was not reported, and forms the focus of this paper.

Study 2

Seven patients on antidepressant treatment (SSRI $n=5$, Venlafaxine $n=2$) were included in this study. All subjects met the DSM-IV criteria for a major depressive episode. The control group consisted of seven medication-free healthy volunteers. Four of the healthy volunteers were recruited and examined previously (Rabiner *et al*, 2000), while the other three were newly recruited for this study. All subjects received two open label [^{11}C]WAY-100635 PET scans (median interval 8 days, range 3–258 days). The first was a baseline scan, while the second was conducted 2 h following 10 mg p.o. of pindolol.

The demographic details of all subjects for both studies are summarized in Table 1.

PET Data Acquisition

PET scans were performed on an ECAT 953B PET camera (CTI/Siemens, Knoxville, TN, USA) (Spinks *et al*, 1992) in three-dimensional mode with dual window scatter correction (Grootoonk *et al*, 1996) and a measured attenuation correction. [^{11}C]WAY-100635 was prepared at the Cyclotron Unit by ^{11}C-carboxylation of a Grignard reagent (McCarron *et al*, 1996), and injected intravenously as a bolus (activity injected 334 ± 69 MBq, mass of WAY 100635 injected 2.85 ± 2.24 μg). In order to assess the influence of pindolol on the metabolism of [^{11}C]WAY-100635, three venous samples were collected at 1000, 2000, and 3000 seconds and percentage of injected [^{11}C]WAY-100635 estimated at each time point. In addition, venous blood samples were taken for estimation of plasma levels of pindolol at the time of the PET scan.

PET Data Analysis

The [^{11}C]WAY-100635 PET scans were analyzed using a reference tissue compartmental model, with the cerebellum as a reference tissue as described previously (Gunn *et al*, 1997, 1998; Lammertsma and Hume, 1996; Rabiner *et al*, 2002a). The reference tissue model allows the estimation of binding potential ($BP=f_2\ B_{AVAIL}/K_D$, where f_2 is the 'free fraction' of the radioligand in the tissue not specifically bound, B_{AVAIL} is the concentration of available binding sites and K_D is the equilibrium dissociation rate constant of the radioligand (Cunningham and Lammertsma, 1994)) and the ratio of radioligand delivery in the region of interest relative to the reference region (R_I). Occupancy of the 5-HT$_{1A}$ receptor sites was inferred as a reduction of BP, and hence

Table 1 Subject Details

						Injected mass (μg)		Injected dose (MBq)	
Study #	**Group**	**Dose of pindolol**	**Sex**	**Age**	**HAM-D**	**Scan 1**	**Scan 2**	**Scan 1**	**Scan 2**
1	SSRIs ($n=4$)	2.5 mg t.i.d. × 1–2 weeks	4 M	41 (24–61)	13 (4–17)	1.4	2.4	281	354
	SSRIs ($n=4$)	5 mg t.i.d. × 1–2 weeks	3 M, 1 F	47 (39–57)	18 (10–29)	5.5	4.3	365	333
2	SSRIs ($n=7$)	10 mg p.o. single dose	7 M	41 (29–57)	13 (6–25)	3.1	3.5	353	349
	DF ($n=7$)	10 mg p.o. single dose	7 M	36 (27–54)	—	1.4	1.9	329	307

B_{AVAIL}, under the assumption that f_2 and K_D remain constant for the two scans.

$$\text{Occupancy} = \frac{BP_{baseline} - BP_{pindolol}}{BP_{baseline}} \times 100 \quad (1)$$

Brain regions were defined as described previously (Rabiner *et al*, 2002a). Briefly, cortical and limbic regions were defined via the warping of a brain region map drawn on an MR image in standard space, onto an individual subject image. The midbrain raphe nuclei (RN) region is a small region readily seen on a [^{11}C]WAY 100635 PET image, but not apparent on an MR image. The raphe region was therefore manually defined as a fixed size region (900 mm^3), on individual subject PET images summed over 20–90 min of the scan. Occupancy was calculated for the 5-HT$_{1A}$ autoreceptor (OCC_{AUTO}, inferred from the occupancy of the midbrain RN) and the 5-HT$_{1A}$ postsynaptic receptor (OCC_{POST} inferred from the average occupancy of the cortical and limbic regions of interest). Preferential Occupancy (PREFocc)was defined as:

$$PREF_{OCC} = OCC_{AUTO} - OCC_{POST} \quad (2)$$

Plasma Data Analysis

Plasma pindolol was measured at the time of injection of the radioligand and at 15 min postradioligand injection. A mean pindolol plasma value was calculated for the first 15 min of the PET scan, and this value was correlated to the occupancy levels achieved.

Statistics

A difference in BP (ΔBP as per Eq. 3)) was calculated for the autoreceptor and postsynaptic regions, from a database of 15 healthy volunteers scanned on two occasions (Rabiner *et al*, 2002a).

$$\Delta BP = \frac{BP_1 - BP_2}{(BP_1 + BP_2)/2} \quad (3)$$

OCC_{AUTO} and OCC_{POST} for the pindolol groups were compared to the ΔBP_{AUTO} and ΔBP_{POST} from the test–retest group using a one-way ANOVA, with *post hoc* testing by a Dunnet's *t*-test (two sided). $PREF_{OCC}$ was compared in the same way to the ΔBP_{auto}–ΔBP_{post} of the test–retest group. In addition, a direct comparison of $PREF_{OCC}$ between the healthy volunteer and the depressed groups of study 2 was performed via a Student's *t*-test.

Changes in the R_I were assessed in the same way as the changes in BP. The change in the percent of parent WAY-100635 following pindolol administration was assessed via a repeated measures ANOVA, with time being the within subject factor, and experimental group being the between subject factor.

[^{11}C]WAY-100635 binding was quantified by the simplified reference tissue model, making it important to examine the reference region (here the cerebellum) for changes in radioligand kinetics that may lead to erroneous conclusions. Although changes in the nonspecific binding of the radioligand in the cerebellum cannot be quantified with a reference region model, a change in the shape of the cerebellar time–activity curves (Cb TACs) would be consistent with alterations in the kinetics of [^{11}C]WAY 100635 nonspecific binding. The Cb TAC for each subjects scan1 and scan 2 were each normalized to their own peak, and the peak normalized TAC for scan 2 subtracted from the corresponding TAC for scan 1, generating a difference TACs (Diff TAC). The same procedure was performed on the 15 subjects of the test–retest group. The Diff TACs of the subjects who received pindolol were compared to the Diff TACs of the test–retest group using a repeated measures ANOVA (time being a within subject factor and group a between subject factor) to assess the effect of pindolol on the time course of the radioligand in the cerebellum.

All statistics were performed on SPSS version 10.1.

RESULTS

Study 1

The data from Study 1 are presented in Table 2. The mean injected dose of [^{11}C]WAY 100635 in Study 1 was 333 ± 65 MBq (mean mass of WAY 100635 injected 3.4 ± 2.8 μg). There were no significant differences in either the injected dose of [^{11}C]WAY 100635 or injected mass of

Table 2 5-HT$_{1A}$ Receptor Occupancy Following Repeated Doses of Pindolol (Study 1)

Subject #	BP_{AUTO} (BP_1, BP_2)	BP_{POST} (BP_1, BP_2)	OCC_{AUTO}	OCC_{POST}	$PREF_{OCC}$	(pindolol) ng/ml	HAM-D	BDI
1 (7.5 mg)	3.07, 2.09	4.19, 3.28	32	22	10	6.6	4	3
2 (7.5 mg)	3.63, 4.23	5.43, 4.68	−17	14	−31	—	16	23
3 (7.5 mg)	2.94, 3.54	3.95, 4.46	−20	−13	−7	13.2	9	7
4 (7.5 mg)	2.60, 2.56	4.00, 3.14	2	21	−19	15.4	21	14
Mean (SD)			0.78 (23.9)	11.0 (16.4)	−11.8 (17.5)	12.7 (2.6)	12.5 (8.3)	11.8 (8.8)
5 (15 mg)	5.56, 3.29	4.89, 2.78	41	43	−2	21.4	11	23
6 (15 mg)	2.43, 2.00	3.07, 2.71	18	12	6	38.6	6	31
7 (15 mg)	3.45, 3.53	5.02, 3.71	−2	26	−28	12.9	19	17
8 (15 mg)	3.15, 2.53	4.23, 3.32	20	22	−2	60.2	3	6
Mean (SD)			19.0 (17.6)	25.7 (13.1)	−6.7 (15.0)	33.6 (22.9)	13.5 (9.4)	19.3 (10.5)

WAY 100635 between the baseline and pindolol scans (injected dose mean difference 21 ± 98 MBq, injected mass mean difference 0.07 ± 2.35 μg). One-way ANOVAs compared pindolol occupancy at the autoreceptor (OCC_{AUTO}) and postsynaptic (OCC_{POST}) receptor sites, as well as preferential occupancy ($PREF_{OCC}$) in the following groups: 15 healthy volunteers Test–Retest (data from Rabiner *et al* (2002a)), four depressed patients following a 7–14-day course of pindolol augmentation (7.5 mg daily), and four depressed patients following a 7–14-day course of pindolol augmentation (15 mg daily). Pindolol had a significant effect on OCC_{POST} ($F_{2,26} = 11.02$, $p = 0.001$) but not OCC_{AUTO} ($F_{2,26} = 2.02$, $p = 0.159$) or $PREF_{OCC}$ ($F_{2,26} = 1.94$, $p = 0.170$). *Post hoc* Dunnett's *t* test (two sided) revealed a significant effect of the 15 mg ($p < 0.001$) but not the 7.5 mg dose ($p = 0.054$) on OCC_{POST}.

An analogous analysis of the R_I values demonstrated no significant effect of pindolol on OCC_{POST} ($F_{2,26} = 0.62$, $p = 0.550$), OCC_{AUTO} ($F_{2,26} = 1.73$, $p = 0.203$), or $PREF_{OCC}$ ($F_{2,26} = 2.77$, $p = 0.087$).

An examination of the differences in cerebellar time–activity curves before and after pindolol administration revealed no effect of pindolol on the binding of [^{11}C]WAY-100635 in the reference region (main effect of group $F_2 = 1.89$, $p = 0.177$). Examination of the metabolism of [^{11}C]WAY 100635 over the period of 1000–3000 s after injection revealed no main effect of time ($F_{1.41} = 3.50$, $p = 0.071$), or group ($F_2 = 0.032$, $p = 0.969$), but a time by group interaction ($F_{2.82} = 4.80$, $p = 0.018$).

Study 2

The data from Study 2 are presented in Table 3. The mean injected dose of [^{11}C]WAY 100635 in study 2 was 335 ± 72 (mean mass of WAY 100635 injected 2.5 ± 1.8 μg). There were no significant differences in either the injected dose of [^{11}C]WAY 100635 or injected mass of WAY 100635 between the baseline and pindolol scans (injected dose mean difference 13 ± 85 MBq, injected mass mean difference -0.51 ± 2.35 μg). One-way ANOVAs compared pindolol occupancy at the autoreceptor (OCC_{AUTO}) and postsynaptic (OCC_{POST}) receptor sites, as well as preferential occupancy ($PREF_{OCC}$) in the following groups: 15 healthy volunteers Test–Retest (data from Rabiner *et al*, 2002a), seven healthy volunteers following a single 10 mg dose of pindolol and seven depressed patients on SSRIs following a single 10 mg dose of pindolol. Pindolol had a significant effect on OCC_{POST} ($F_{2,26} = 13.64$, $p < 0.001$), OCC_{AUTO} ($F_{2,26} = 11.68$, $p < 0.001$), and $PREF_{OCC}$ ($F_{2,26} = 6.40$, $p = 0.005$). *Post hoc* Dunnett's *t* tests (two sided) revealed that pindolol 10 mg single dose had a significant effect in both the patients and the healthy volunteers on OCC_{POST} ($p < 0.001$ and $p = 0.005$ respectively) and the OCC_{AUTO} ($p < 0.009$ and < 0.001 respectively). By contrast, $PREF_{OCC}$ was significantly different only for the healthy volunteers ($p = 0.005$), but not for patients ($p = 0.994$).

An analogous analysis of the R_I values demonstrated no significant effect of pindolol on OCC_{POST} ($F_{2,26} = 0.25$, $p = 0.779$), but both OCC_{AUTO} ($F_{2,26} = 4.80$, $p = 0.017$) and $PREF_{OCC}$ ($F_{2,26} = 4.38$, $p = 0.023$) were significantly different. *Post hoc* Dunnett's *t* test (two sided) revealed no significant effects in either of the groups, though the patient group approached significance for both OCC_{AUTO} ($p = 0.054$) and $PREF_{OCC}$ ($p = 0.051$).

An examination of the differences in cerebellar [^{11}C]WAY-100635 time–activity curves before and after pindolol administration revealed no effect of pindolol on the time course of [^{11}C]WAY-100635 in the reference region

Table 3 5-HT$_{1A}$ Receptor Occupancy Following a Single Dose of Pindolol (Study 2)

Subject #	BP_{AUTO} (BP_1, BP_2)	BP_{POST} (BP_1, BP_2)	OCC_{AUTO}	OCC_{POST}	$PREF_{OCC}$	(pindolol)	HAM-D	BDI
Healthy volunteers								
1	3.76, 2.74	3.22, 3.09	27	4	23	11.2	—	—
2	4.11, 2.05	3.35, 2.62	50	22	28	23.2	—	—
3	4.24, 2.40	4.19, 3.74	43	11	32	14.9	—	—
4	4.15, 2.35	3.62, 2.93	43	19	24	N/A	—	—
5	5.24, 2.80	4.38, 3.08	47	30	17	24.3	—	—
6	2.82, 1.99	3.02, 2.53	29	16	13	14.9	—	—
7	4.32, 4.09	3.87, 4.26	5	−10	15	2.4	—	—
Mean (SD)			35.0 (15.7)	12.4 (13.1)	22.6 (7.7)	13.4 (8.5)	—	—
Patients on SSRIs								
8	3.72, 2.74	4.40, 2.81	26	36	−10	52.3	16	20
9	3.08, 2.63	4.18, 3.79	15	9	6	33.3	12	20
10	3.62, 2.67	4.76, 3.91	26	18	8	5.4	3	5
11	3.78, 2.57	4.40, 3.51	32	20	12	17.2	N/A	9
12	3.99, 4.11	3.56, 3.54	−3	0	−3	55.0	8	7
13	3.98, 2.64	3.87, 3.26	34	16	18	12.7	12	11
14	3.57, 2.94	4.72, 3.42	18	27	−9	47.6	25	33
Mean (SD)			21.0 (12.7)	18.1 (11.6)	2.9 (10.8)	29.7 (22.8)	12.7 (7.5)	15.0 (9.9)

(main effect of group $F_2 = 1.358$, $p = 0.703$). Examination of the metabolism of [^{11}C]WAY 100635 over the period of 1000–3000 s after injection revealed no main effect of time ($F_{1.10} = 0.225$, $p = 0.665$), or a time by group interaction time ($F_{2.20} = 0.661$, $p = 0.544$). There was however a main effect of group ($F_2 = 12.24$, $p = 0.001$). *Post hoc* tests revealed a significant increase in percent of parent [^{11}C]WAY 100635 in the depressed patients on SSRIs following a single 10 mg dose of pindolol ($p = 0.011$), but not in healthy volunteers.

Preferential Occupancy

Overall, these results demonstrate a preferential occupancy of the 5-HT$_{1A}$ autoreceptor compared to the postsynaptic receptor by pindolol, in healthy volunteers, but not in depressed patients on SSRIs (Figure 1). The loss of preferential occupancy, in depressed patients on SSRI treatment is confirmed by the comparison of $PREF_{OCC}$ in healthy volunteers (mean $PREF_{OCC} = 22.6 \pm 7.7\%$) and depressed patients following a 10 mg single dose of pindolol (mean $PREF_{OCC} = 2.9 \pm 10.8\%$, Student's $t = 3.94$, $df = 12$, $p = 0.002$).

Pindolol Plasma Levels

Plasma pindolol levels were measured as previously detailed (Rabiner *et al*, 2000). There were no significant correlations between the plasma pindolol levels and either autoreceptor, postsynaptic receptor or preferential occupancy (Pearson's r; -0.13 ($p = 0.58$), 0.18 ($p = 0.45$) and -0.33 ($p = 0.16$) respectively). In study 2, the mean plasma pindolol concentration was notably higher in the patient (29.7 ± 22.8 ng/ml) as opposed to the healthy volunteer group (13.4 ± 8.6 ng/ml), though this difference was not statistically significant ($t = -1.74$, $df = 7.87$, $p = 0.12$). The higher plasma pindolol levels did not lead to a significant difference in either the autoreceptor or postsynaptic receptor occupancy ($t = 1.83$, $df = 12$, $p = 0.09$, and $t = -0.86$, $df = 12$, $p = 0.41$ respectively).

DISCUSSION

Pindolol is a mixed β-adrenergic/5-HT$_{1A}$ partial agonist (Clark *et al*, 1982; Clifford *et al*, 1998; Frishman *et al*, 1979; Hjorth and Carlsson, 1986; Meltzer and Maes, 1996; Newman-Tancredi *et al*, 1998; Sanchez *et al*, 1996; Sprouse *et al*, 2000) in several *in vivo* and *in vitro* assays, which has been proposed to accelerate and augment the antidepressant effects of SSRIs via the blockade of 5-HT$_{1A}$ autoreceptors (Artigas, 1993; Blier and de Montigny, 1994; Hjorth and Sharp, 1993), though an effect through its adrenergic activity cannot be excluded. The 5-HT$_{1A}$ receptor is a G-protein coupled receptor (GPCR) with seven trans-membrane domains, and exists as both a somatodendritic autoreceptor expressed on the 5-HT cell bodies and dendrites in the midbrain RN (Riad *et al*, 2000), and a postsynaptic heteroreceptor expressed in cortical and limbic areas (Hall *et al*, 1997; Pazos *et al*, 1987; Pike *et al*, 1996), on neuronal soma, especially perisynaptically. 5-HT$_{1A}$ receptors are also present on astroglia (Azmitia *et al*, 1996), though the density of non-neuronal 5-HT$_{1A}$ receptors may be considerably lower (Kia *et al*, 1996; Verge *et al*, 1986). The 5-HT$_{1A}$ autoreceptors control the release of 5-HT in the forebrain projection areas (Sharp *et al*, 1989; Sprouse and Aghajanian, 1987), and the therapeutic action of

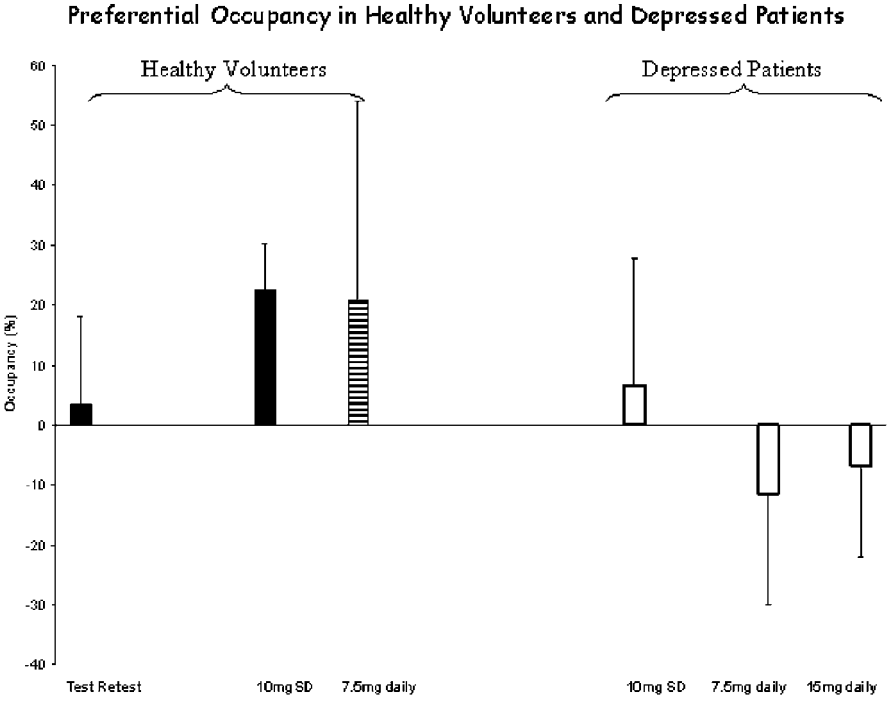

Figure 1 Preferential occupancy of 5-HT$_{1A}$ autoreceptors by pindolol. Mean $\pm$ SD of healthy volunteers (solid bars) and depressed patients (open bars). Striped bar represents healthy volunteer data from Martinez *et al* (2001).

selective SSRIs has been hypothesized to depend on the desensitization of these autoreceptors (Artigas, 1993; Blier and de Montigny, 1994; Hjorth and Sharp, 1993).

Autoreceptor desensitization leads to a decrease in the inhibitory control of serotonergic neuronal activity by the 5-HT$_{1A}$ autoreceptors, and therefore an enhanced 5-HT neurotransmission (Artigas *et al*, 1996). The proposal to accelerate, and/or augment the antidepressant actions of SSRIs via a blockade of RN autoreceptors (Artigas, 1993; Hjorth, 1993; Hjorth and Sharp, 1993), depends therefore on significant pindolol binding to the 5-HT$_{1A}$ autoreceptor site, but has been criticized on the grounds that pindolol would also bind to the postsynaptic 5-HT$_{1A}$ site and therefore counteract any proposed beneficial effects of an increase in forebrain 5-HT (Sprouse *et al*, 2000). While the receptor subtype through which 5-HT exerts its antidepressant action is not certain, considerable evidence points to postsynaptic 5-HT$_{1A}$ receptors being important in this regard. Though the subsensitivity of postsynaptic 5-HT$_{1A}$ receptors in depressed patients has been debated (Cowen *et al*, 1994; Meltzer and Maes, 1995), 5-HT$_{1A}$ postsynaptic heteroreceptors are activated by long-term antidepressant treatment (Blier *et al*, 1987, 1990; de Montigny and Aghajanian, 1978; Haddjeri *et al*, 1998), while the antidepressant actions of SSRIs may depend on the enhanced levels of synaptic 5-HT acting on these receptors (Blier *et al*, 1987; De Vry, 1995). As a blockade of postsynaptic 5-HT$_{1A}$ receptors may block the beneficial effects of raised 5-HT produced by 5-HT$_{1A}$ autoreceptor blockade, compounds blocking the two receptor populations equally may not be effective augmenting agents. Preferential occupancy of the 5-HT$_{1A}$ autoreceptor would therefore make pindolol an attractive candidate for a role in accelerating/augmenting antidepressant effects of SSRIs.

The fact of preferential binding by pindolol seems well established, but the mechanism is uncertain, with regional differences in the affinity status of the 5-HT$_{1A}$ receptor being a plausible hypothesis (Martinez *et al*, 2001; Rabiner *et al*, 2000). The extended ternary model of ligand-receptor binding (Samama *et al*, 1993) posits the receptor to exist in several conformational states with agonists binding preferentially to, and stabilizing some states selectively, and promoting the formation of the ternary receptor–ligand–G-protein complex while antagonists have equal affinity for all conformational states. As pindolol is a partial agonist it may be expected to bind preferentially to the 'high-affinity' (R^*), compared to the 'low-affinity' (R) receptor conformation. If the ratio of R^* sites is higher for the autoreceptor compared to the postsynaptic receptor, then pindolol will appear to have a preferential occupancy of the autoreceptor compared to the postsynaptic receptor, when examined with an antagonist radioligand.

Considerable evidence exists for differences in the functional response of RN as opposed to the cortical and limbic 5-HT$_{1A}$ receptors to pharmacological challenges (Romero *et al*, 1996; Sinton and Fallon, 1988), although not all studies have come to this conclusion (Corradetti *et al*, 1998). The most common explanation given is that the RN 5-HT$_{1A}$ receptors possess greater 'receptor reserve' than the cortical and limbic areas (Jolas *et al*, 1995; Meller *et al*, 1990; Yocca *et al*, 1992). The mechanistic Whaley model of GPCR–Agonist–G-protein interaction (Whaley *et al*, 1994) is equivalent to the earlier empirical Furchgott model (1966) (see Clark *et al*, 1999 for review) and renders the concept of 'receptor reserve' as 'both misleading and irrelevant' (Clark *et al*, 1999). An increased ratio of 'high affinity' sites, would however, provide a higher concentration of receptors that can be acted upon effectively by agonists, therefore increasing the efficacy of a partial agonist (Clark *et al*, 1999). Such a mechanism would provide an explanation for compounds with 5-HT$_{1A}$ agonist effects, having greater efficacy in the RN compared to the postsynaptic areas such as the hippocampus (Andrade and Nicoll, 1987; Fabre *et al*, 1997; Greuel and Glaser, 1992; Hjorth and Sharp, 1990; Sprouse and Aghajanian, 1988).

Alterations in the functional status of the 5-HT$_{1A}$ receptor may be caused either by the antidepressant treatment or the pathophysiology of depression. Reductions in [^{11}C]WAY-100635 binding to human 5-HT$_{1A}$ auto and heteroreceptors in depressed patients have been reported in several *in vivo* studies (Sargent *et al*, 2000; Drevets *et al*, 1999; Parsey *et al*, 2002) and have been interpreted as a modest reduction in 5-HT$_{1A}$ receptor numbers in depressed patients. The functional significance of a 10–15% reduction in receptor number in these patients is unclear, especially considering recent studies which demonstrated a 70% blockade of the 5-HT$_{1A}$ receptor in healthy volunteers without significant side effects (Rabiner *et al*, 2002b). On the other hand, the functional consequences of an alteration of receptor–G-protein coupling may be much more pronounced, but not apparent in an examination using an antagonist radioligand, [^{11}C]WAY 100635, to determine receptor density.

Chronic agonist stimulation, such as that induced by long-term administration of SSRIs, causes a desensitization of the somatodendritic 5-HT$_{1A}$ receptors, but not the postsynaptic receptors (see Hensler, 2003 for a review). Mechanisms at several levels may explain receptor desensitization without a downregulation of receptor numbers. Receptor level mechanisms, such as regulation at the level of G-protein coupled receptor kinases (GRKs), arrestin binding, and endocytosis appear to dominate receptor desensitization. Downstream effects, such as agonist-induced phosphorylation of G-proteins, phospholipase C, and adenylate cyclase, may become important as the duration of stimulation increases. The reason for the differential effects of SSRIs on 5-HT$_{1A}$ desensitization in the RN compared to the postsynaptic receptors is unclear, but may relate to the differential coupling of these receptors to G-proteins, and effector mechanisms. For instance, while frontal cortex 5-HT$_{1A}$ receptors are coupled to both Go and Gi_3, those in the RN are coupled to Gi_3 only (Hensler, 2003).

If we hypothesize that preferential occupancy is a consequence of differing proportions of 'high-affinity' and 'low-affinity' sites in the RN compared to the cortical regions, then the loss of preferential occupancy in depressed patients on chronic SSRIs would imply that in these patients there is a change in the proportions of 'high-affinity' and 'low-affinity' sites in the autoreceptor and the postsynaptic receptor regions. In fact, the decrease in the autoreceptor occupancy, with the relative preservation of postsynaptic receptor occupancy seen in the patients in this study (see Table 3), implies that the loss of preferential occupancy is a

consequence of the loss of 'high-affinity' sites in the RN. This loss of 'high-affinity' sites can be a consequence of either the SSRI treatment or that of the illness. From the discussion above, chronic SSRI treatment may be expected to lead to receptor desensitization because of chronic stimulation of the receptor by the endogenous agonist (5-HT), as discussed above. The effects of depression on the efficacy status of 5-HT$_{1A}$ receptors are less well documented. Chronic ultramild stress (CUMS), a plausible model of depression, causes a desensitization to the effects of the 5-HT$_{1A}$ partial agonist ipsapirone in mice (Lanfumey *et al*, 1999). In addition, other forms of stress as well as the application of corticosterone to brain slices have been shown to desensitize RN 5-HT$_{1A}$ autoreceptors (Laaris *et al*, 1995, 1999).

An unpredicted result of this study was a significant negative correlation (Spearman's $\rho = -0.728$, $N = 14$, $p = 0.003$) between the severity of depression (as judged by the HAM-D score) and the PREF$_{OCC}$ (Figure 2). The correlation between the Beck's Depression Inventory (BDI) and PREF$_{OCC}$ did not reach significance (Spearman's $\rho = -0.439$, $N = 15$, $p = 0.102$). The BDI consists of Factor 1 (cognitive and mood items) and Factor 2 (somatic items) (Schotte *et al*, 1997). PREF$_{OCC}$ correlates significantly with the somatic items of the BDI (Factor 2, Spearman's $\rho = -0.539$, $N = 15$, $p = 0.038$) but not the cognitive and mood items BDI (Factor 1, Spearman's $\rho = -0.472$, $N = 15$, $p = 0.075$). These findings support the view that depressive illness affects pindolol occupancy and, by implication, the functioning of the RN 5-HT$_{1A}$ autoreceptors.

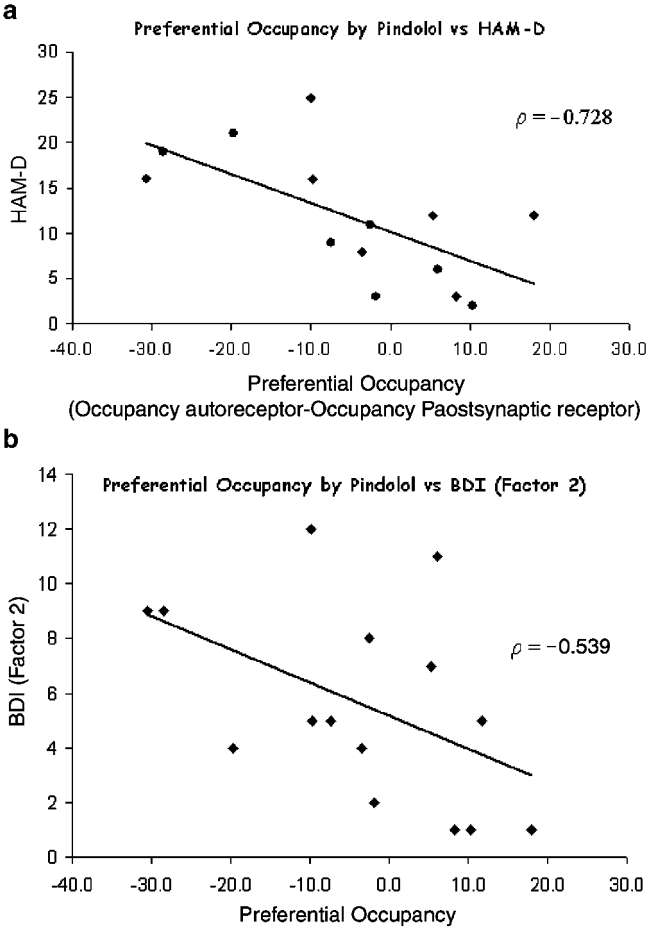

Figure 2 Correlation of preferential occupancy and severity of depression: (a) correlation of PREF$_{OCC}$ with HAM-D. (b) correlation of PREF$_{OCC}$ with BDI, Factor 2.

If the desensitization of RN 5-HT$_{1A}$ receptors is considered to be a necessary effect of SSRI treatment, one would expect that patients who respond to treatment (and therefore have lower HAM-D scores) will have a lower degree of PREF$_{OCC}$. Examination of Figure 2, on the other hand, reveals the opposite, meaning that patients with a lower HAM-D score have *higher* PREF$_{OCC}$ than those with more severe symptoms. This finding raises the possibility that the loss of PREF$_{OCC}$ may be a result of the illness, and as such is an index of the severity of depression, rather than an index of the effects of SSRI treatment. If the attenuation of PREF$_{OCC}$ is an index of depression severity, it could provide a valuable surrogate in the investigation of the underlying mechanisms of depression. However, the attenuation of PREF$_{OCC}$ may represent an interaction of two separate mechanisms, with both SSRI mediated effects and the direct effects of depression playing a role.

A recent review (Blier, 2003) concluded that the evidence for acceleration of antidepressant effects by pindolol is much more convincing than an augmentation effect. If the attenuation of PREF$_{OCC}$ is, at least in part, a consequence of the effect of chronic SSRI administration, and preferential occupancy is important for the mechanism of action of pindolol, in augmenting antidepressant actions of SSRIs, it may be expected that patients who receive pindolol at the start of their treatment (concurrent with the SSRIs) will have a better clinical response than those that receive pindolol after a prolonged period of antidepressant treatment. Similarly, if the severity of depression correlates negatively with PREF$_{OCC}$, then the less severe patients will benefit from pindolol augmentation more than the more chronic, treatment resistant patients. These suppositions have some support from clinical practice.

Further studies are needed to elucidate the contributions of SSRIs and depression severity, in the attenuation of PREF$_{OCC}$ by pindolol; however, the discussion above indicates that while augmentation of antidepressant effects of SSRIs by pindolol may be an efficacious intervention, only a subset of patients (antidepressant free subjects with mild to moderate depression severity) may benefit. In addition, as we have discussed previously (Rabiner *et al*, 2001), the dose of pindolol commonly used in clinical trials, 2.5 mg t.i.d., is inadequate to produce a consistent occupancy of the 5-HT$_{1A}$ autoreceptor in depressed patients on SSRIs, a factor contributing to the variability of clinical response in these trials (Rabiner *et al*, 2001). This finding, supported by pindolol occupancy studies in healthy volunteers (Martinez *et al*, 2001; Rabiner *et al*, 2000), indicates that the utility of pindolol in the clinic may be limited, because an increase in dose will also lead to an increased incidence of β-adrenergic side effects. Novel compounds, selective for the 5-HT$_{1A}$ receptor, and having a similar weak partial-agonist profile as pindolol, may prove to be more successful. Nevertheless, pindolol will remain useful as a tool compound in the investigation of serotonergic neurotransmission in humans *in vivo*.

Limitations of This Study

We examined pindolol occupancy in a small group of depressed patients on SSRI treatment, which does not allow us to differentiate the effects of illness form the effects of treatment. Future studies will need to examine drug-free depressed patients, and healthy volunteers following several weeks of SSRI treatment, in order to disentangle these components. In addition, this work combines two different pindolol regimes, a single 10 mg dose, and repeat dosages of 7.5 and 15 mg daily. While these differences in pindolol regimes may cause variability in the results, the two groups appear comparable, as repeated administration of 7.5 mg of pindolol daily to healthy volunteers (Martinez *et al*, 2001) produced preferential occupancy similar to that produced by a single dose of 10 mg of pindolol (Figure 1). All the results reported above remain significant when plasma pindolol concentration is introduced as a covariate (data not shown).

It is necessary to consider whether the preferential occupancy induced by pindolol may be a methodological artefact of the PET procedure, rather than reflecting a true increase in binding by pindolol at the RN. The various methodological caveats, such as partial volume effects, were discussed by Martinez *et al* (2001), who concluded that it was unlikely that preferential occupancy is artefactual. Our data as well as data from other units indicate that high occupancies of the 5-HT$_{1A}$ receptors (up to 70%) can be reached by antagonist compounds without preferential occupancy being found (Andree *et al*, 2003; Rabiner *et al*, 2002b), supporting the view that preferential occupancy is not due to a non-linearity in partial volume effects.

We found some evidence of a decrease in delivery of the radioligand in the RN compared to cortical regions in healthy volunteers treated with pindolol, but not in patients. Although the simplified reference tissue model assumes that BP and R_I are independent parameters, some co-dependence has been noted previously. To examine the possibility that decreases in R_I could lead to decreases in BP (and hence artifactual occupancy), we conducted simulations in which R_I was varied and the effect on BP was estimated. The results, presented in Figure 3, indicate that a decline in R_I does not lead to a decrease in the estimated BP (if anything the effect is in the opposite direction). Preferential occupancy effect has also been found by Martinez using an alternative quantification method (arterial plasma derived input function (Martinez *et al*, 2001)).

Finally, a main effect of treatment was found in the examination of the effects of pindolol on the metabolism of [^{11}C]WAY 100635, with the depressed patients who received a 10 mg single dose having a significantly slower clearance of the radioligand from the plasma. This may be due to an interaction between pindolol and the liver enzymes metabolizing [^{11}C]WAY 100635. However, changes in the profile of the radioligand in the plasma are likely to produce global effects on radioligand binding, rather than the regional differences seen in above, and we do not expect these changes to account for the loss of preferential occupancy we found.

In conclusion, we have shown that preferential occupancy of the 5-HT$_{1A}$ autoreceptors by pindolol is attenuated in depressed patients on SSRIs. This phenomenon may be an effect of SSRI treatment, or of the pathophysiology of depression, and as such may have important implications in clinical practice of antidepressant treatment augmentation.

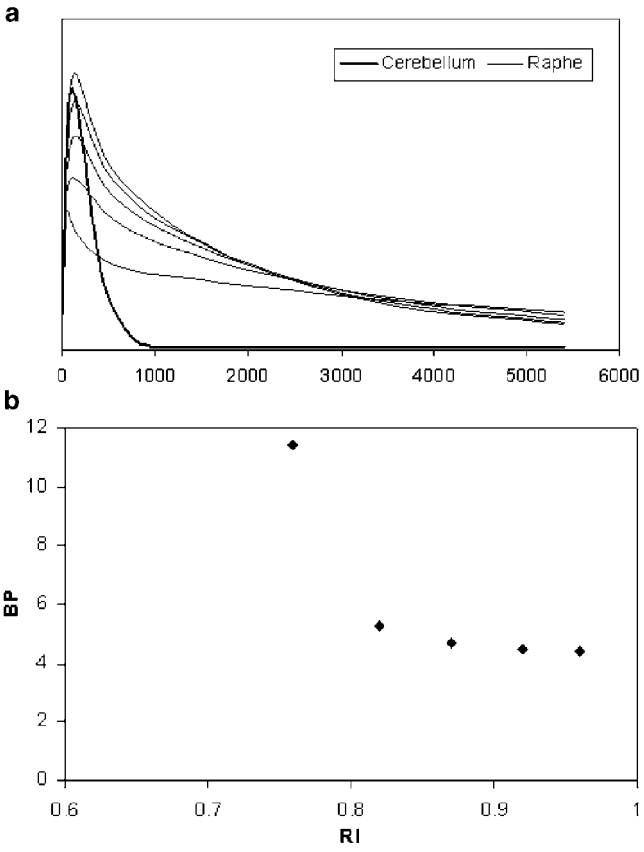

Figure 3 Simulation of the effects of a change in R_I on the value of BP estimated by a simplified reference tissue model. (a) Simulated time–activity curves for the reference region (thick line) and five runs for a region of interest (thin line). (b) Results of R_I and BP estimation by a simplified reference region model, at various simulated levels of R_I. The simulations were conducted using a compartmental model with two tissue compartments in the target tissue and one compartment in the reference tissue. The following values of the kinetic constants (in m^{-1}, $k_1 = 0.1$, $k_2 = 0.5$, $k_3 = 0.25$, $k_4 = 0.05$) were used in the target tissue. The values for the reference tissue were identical, except that k_3 and k_4 were set to 0. Decreases in R_I were simulated as decreases in k_1 and k_2 in the target tissue.

ACKNOWLEDGEMENTS

We thank the staff of the MRC Clinical Sciences PET unit, for excellent technical support, and especially Joanne Holmes, Andy Blyth, Safiye Osman, Dave Turton, and Ray Khan. We would also like to thank Michael Clement and Mike Franklin of the Psychopharmacology Research Unit, University of Oxford, for the determination of plasma pindolol levels.

REFERENCES

Adell A, Artigas F (1991). Differential effects of clomipramine given locally or systemically on extracellular 5-hydroxytrypta-

mine in raphe nuclei and frontal cortex. *Naunyn-Schmiedebergs Arch Pharmacol* **343**: 237–244.

Albert PR, Zhou QY, Van Tol HH, Bunzow JR, Civelli O (1990). Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine1A receptor gene. *J Biol Chem* **265**: 5825–5832.

Andrade R, Nicoll RA (1987). Novel anxiolytics discriminate between postsynaptic serotonin receptors mediating different physiological responses on single neurons of the rat hippocampus. *Naunyn-Schmiedebergs Arch Pharmacol* **336**: 5–10.

Andree B, Hedman A, Thorberg SO, Nilsson D, Halldin C, Farde L (2003). Positron emission tomographic analysis of dose-dependent NAD-299 binding to 5-hydroxytryptamine-1A receptors in the human brain. *Psychopharmacology* **167**: 35–45.

Artigas F (1993). 5-HT and antidepressants: new views from microdialysis studies. *Trends Pharmacol Sci* **14**: 262.

Artigas F, Celada P, Laruelle M, Adell A (2001). How does pindolol improve antidepressant action? *Trends Pharmacol Sci* **22**: 224–228.

Artigas F, Romero L, Montigny de C, Blier P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT1A antagonists. *Trends Neurol Sci* **19**: 378–383.

Azmitia EC, Gannon PJ, Kheck NM, Whitaker-Azmitia PM (1996). Cellular localization of the 5-HT1A receptor in primate brain neurons and glial cells. *Neuropsychopharmacology* **14**: 35–46.

Blier P (2003). The pharmacology of putative early-onset antidepressant strategies. *Eur Neuropsychopharmacol* **13**: 57–66.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* **15**: 220–226.

Blier P, de Montigny C, Chaput Y (1987). Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J Clin Psychopharmacol* **7**: 24S–35S.

Blier P, de Montigny C, Chaput Y (1990). A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J Clin Psychiatry* **51**(Suppl): 14–20; discussion 21.

Castro ME, Harrison PJ, Pazos A, Sharp T (2000). Affinity of (+/−)-pindolol, (−)-penbutolol, and (−)-tertatolol for pre- and postsynaptic serotonin 5-HT(1A) receptors in human and rat brain. *J Neurochem* **75**: 755–762.

Chaput Y, de Montigny C, Blier P (1991). Presynaptic and postsynaptic modifications of the serotonin system by long-term antidepressant treatments: electrophysiological studies in the rat brain. *Neuropsychopharmacology* **5**: 219–229.

Clark BJ, Menninger K, Bertholet A (1982). Pindolol—the pharmacology of a partial agonist. *Br J Clin Pharmacol* **13**: 149S–158S.

Clark RB, Knoll BJ, Barber R (1999). Partial agonists and G protein-coupled receptor desensitization. *Trends Pharmacol Sci* **20**: 279–286.

Clifford EM, Gartside SE, Umbers V, Cowen PJ, Hajos M, Sharp T (1998). Electrophysiological and neurochemical evidence that pindolol has agonist properties at the 5-HT1A autoreceptor *in vivo*. *Br J Pharmacol* **124**: 206–212.

Corradetti R, Laaris N, Hanoun N, Laporte AM, Le Poul E, Hamon M *et al* (1998). Antagonist properties of (−)-pindolol and WAY 100635 at somatodendritic and postsynaptic 5-HT1A receptors in the rat brain. *Br J Pharmacol* **123**: 449–462.

Cowen PJ, Power AC, Ware CJ, Anderson IM (1994). 5-HT1A receptor sensitivity in major depression. A neuroendocrine study with buspirone. *Br J Psychiatry* **164**: 372–379.

Cunningham VJ, Lammertsma AA (1994). Radioligand studies in brain: kinetic analysis of PET data. *Med Chem Res* **5**: 79–96.

de Montigny C, Aghajanian GK (1978). Tricyclic antidepressants: long-term treatment increases responsivity of rat forebrain neurons to serotonin. *Science* **202**: 1303–1306.

De Vry J (1995). 5-HT$_{1A}$ receptor agonists: recent developments and controversial issues. *Psychopharmacology (Berlin)* **121**: 1–26.

Drevets WC, Frank E, Price JC, Kupfer DJ, Holt D, Greer PJ *et al* (1999). PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry* **46**: 1375–1387.

Fabre V, Boni C, Mocaer E, Lesourd M, Hamon M, Laporte AM (1997). [3H]Alnespirone: a novel specific radioligand of 5-HT1A receptors in the rat brain. *Eur J Pharmacol* **337**: 297–308.

Frishman W, Kostis J, Strom J, Hossler M, Elkayam U, Goldner S *et al* (1979). Clinical pharmacology of the new beta-adrenergic blocking drugs. Part 6. A comparison of pindolol and propranolol in treatment of patients with angina pectoris. The role of intrinsic sympathomimetic activity. *Am Heart J* **98**: 526–535.

Furchgott RF (1966) In Harper NJ, Simmonds AB (eds). *Advances in Drug Research*. Academic Press: New York. pp 21–55.

Greuel JM, Glaser T (1992). The putative 5-HT1A receptor antagonists NAN-190 and BMY 7378 are partial agonists in the rat dorsal raphe nucleus *in vitro*. *Eur J Pharmacol* **211**: 211–219.

Grootoonk S, Spinks TJ, Sashin D, Spyrou NM, Jones T (1996). Correction for scatter in 3D brain PET using a dual energy window method. *Phys Med Biol* **41**: 2757–2774.

Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997). Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *Neuroimage* **6**: 279–287.

Gunn RN, Sargent PA, Bench CJ, Rabiner EA, Osman S, Pike VW *et al* (1998). Tracer kinetic modelling of the 5-HT$_{1A}$ receptor ligand [*carbonyl*-^{11}C]WAY-100635 for PET. *Neuroimage* **8**: 426–440.

Haddjeri N, Blier P, de Montigny C (1998). Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT1A receptors. *J Neurosci* **18**: 10150–10156.

Hall H, Lundkvist C, Halldin C, Farde L, Pike VW, McCarron JA *et al* (1997). Autoradiographic localization of 5-HT1A receptors in the post-mortem human brain using [3H]WAY-100635 and [11C]way-100635. *Brain Res* **745**: 96–108.

Hensler JG (2003). Regulation of 5-HT1A receptor function in brain following agonist or antidepressant administration. *Life Sci* **72**: 1665–1682.

Hirani E, Opacka-Juffry J, Gunn R, Khan I, Sharp T, Hume S (1999). Pindolol occupancy of 5-HT$_{1A}$ receptors measured *in vivo* using small animal positron emission tomography with carbon-11 labelled WAY 100635. *Synapse* **36**: 330–341.

Hjorth S (1993). Serotonin 5-HT1A autoreceptor blockade potentiates the ability of the 5-HT reuptake inhibitor Citalopram to increase nerve terminal output of 5-HT *in vivo*: A microdialysis study. *J Neurochem* **60**: 776–779.

Hjorth S, Carlsson A (1986). Is pindolol a mixed agonist-antagonist at central serotonin (5-HT) receptors? *Eur J Pharmacol* **129**: 131–138.

Hjorth S, Sharp T (1990). Mixed agonist/antagonist properties of NAN-190 at 5-HT1A receptors: behavioural and *in vivo* brain microdialysis studies. *Life Sci* **46**: 955–963.

Hjorth S, Sharp T (1993). *In vivo* microdialysis evidence for central serotonin1A and serotonin1B autoreceptor blocking properties of the beta-adrenoceptor antagonist (−)-penbutolol. *J Pharmacol Exp Therapeut* **265**: 707–712.

Invernizzi R, Belli S, Samanin R (1992). Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. *Brain Res* **584**: 322–324.

Invernizzi R, Carli M, DiClimente A, Samanin R (1991). Administration of 8-hydroxy-2-(di-n-propylamino) tetralin in raphe nuclei dorsalis and medialis reduces serotonin synthesis in the rat brain: differences in potency and regional sensitivity. *J Neurochem* **56**: 243–247.

Jolas T, Schreiber R, Laporte AM, Chastanet M, De Vry J, Glaser T *et al* (1995). Are postsynaptic 5-HT1A receptors involved in the

anxiolytic effects of 5-HT1A receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? *J Pharmacol Exp Therapeut* **272**: 920–929.

Kia HK, Brisorgueil MJ, Hamon M, Calas A, Verge D (1996). Ultrastructural localization of 5-hydroxytryptamine1A receptors in the rat brain. *J Neurosci Res* **46**: 697–708.

Laaris N, Haj-Dahmane S, Hamon M, Lanfumey L (1995). Glucocorticoid receptor-mediated inhibition by corticosterone of 5-HT1A autoreceptor functioning in the rat dorsal raphe nucleus. *Neuropharmacology* **34**: 1201–1210.

Laaris N, Le Poul E, Laporte AM, Hamon M, Lanfumey L (1999). Differential effects of stress on presynaptic and postsynaptic 5-hydroxytryptamine-1A receptors in the rat brain: an *in vitro* electrophysiological study. *Neuroscience* **91**: 947–958.

Lammertsma AA, Hume SP (1996). Simplified reference tissue model for PET receptor studies. *Neuroimage* **4**: 153–158.

Lanfumey L, Pardon MC, Laaris N, Joubert C, Hanoun N, Hamon M *et al* (1999). 5-HT1A autoreceptor desensitization by chronic ultramild stress in mice. *Neuroreport* **10**: 3369–3374.

Martinez D, Hwang D, Mawlawi O, Slifstein M, Kent J, Simpson N *et al* (2001). Differential occupancy of somatodendritic and postsynaptic 5HT(1A) receptors by pindolol: a dose-occupancy study with [11C]WAY 100635 and positron emission tomography in humans. *Neuropsychopharmacology* **24**: 209–229.

McCarron JA, Turton DR, Pike VW, Poole KG (1996). Remotely controlled production of the 5-HT$_{1A}$ receptor radioligand, [*carbonyl*-^{11}C]WAY-100635, via 11C-carboxylation of an immobilized Gringard reagent. *J Labelled Compd Radiopharmaceut* **38**: 941–953.

Meller E, Goldstein M, Bohmaker K (1990). Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. *Mol Pharmacol* **37**: 231–237.

Meltzer HY, Maes M (1995). Effects of ipsapirone on plasma cortisol and body temperature in major depression. *Biol Psychiatry* **38**: 450–457.

Meltzer HY, Maes M (1996). Effect of pindolol on hormone secretion and body temperature: partial agonist effects. *J Neural Transmission* **103**: 77–88.

Newman-Tancredi A, Chaput C, Gavaudan S, Verriele L, Millan MJ (1998). Agonist and antagonist actions of (−)pindolol at recombinant human serotonin 1A (5-HT$_{1A}$) receptors. *Neuropsychopharmacology* **18**: 395–398.

Parsey RV, Oquendo MA, Simpson NR, Huang Y, Van Heertum R, Arango V *et al* (2002). Altered serotonin 1A binding in major depression: A [C-11]WAY100635 PET study. *Biol Psychiatry* **8S**: 106S.

Pazos A, Probst A, Palacios JM (1987). Serotonin receptors in the human brain-III. Autoradiographic mapping of serotonin-1 receptors. *Neuroscience* **21**: 97–122.

Pike VW, McCarron JA, Lammertsma AA, Osman S, Hume SP, Sargent PA *et al* (1996). Exquisite delineation of 5-HT$_{1A}$ receptors in human brain with PET and [*carbonyl*-^{11}C]-WAY100635. *Eur J Pharmacol* **301**: R5–R7.

Rabiner EA, Bhagwagar Z, Gunn RN, Sargent PA, Bench CJ, Cowen PJ *et al* (2001). Pindolol augmentation of selective serotonin reuptake inhibitors: PET evidence that the dose used in clinical trials is too low. *Am J Psychiatry* **158**: 2080–2082.

Rabiner EA, Gunn RN, Castro ME, Sargent PA, Cowen PJ, Koepp MJ *et al* (2000). β-blocker binding to human 5-HT$_{1A}$ receptors *in vivo* and *in vitro*: implications for antidepressant therapy. *Neuropsychopharmacology* **23**: 285–293.

Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD *et al* (2002a). A database of [(11)C]WAY-100635 binding to 5-HT(1A) receptors in normal male volunteers: normative data and relationship to methodological, demographic, physiological, and behavioral variables. *Neuroimage* **15**: 620–632.

Rabiner EA, Wilkins MR, Turkheimer F, Gunn RN, de Haes JU, de Vries M *et al* (2002b). 5-Hydroxytryptamine1A receptor occupancy by novel full antagonist 2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzdioxyn-5-yl)-1-piperazinyl]butyl]-1,2-benzisothiazol-3-(2H)-one-1,1-dioxide: a[11C][O-methyl-3H]-*N*-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635) positron emission tomography study in humans. *J Pharmacol Exp Therapeut* **301**: 1144–1150.

Radja F, Daval G, Hamon M, Verge D (1992). Pharmacological and physicochemical properties of pre- *versus* postsynaptic 5-hydroxytryptamine1A receptor binding sites in the rat brain: a quantitative autoradiographic study. *J Neurochem* **58**: 1338–1346.

Raurich A, Mengod G, Artigas F, Cortes R (1999). Displacement of the binding of 5-HT$_{1A}$ receptor ligands to pre- and postsynaptic receptors by (−)pindolol. A comparative study in rodent, primate and human brain. *Synapse* **34**: 68–76.

Riad M, Garcia S, Watkins KC, Jodoin N, Doucet E, Langlois X *et al* (2000). Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J Comp Neurol* **417**: 181–194.

Romero L, Bel N, Artigas F, de Montigny C, Blier P (1996). Effect of pindolol on the function of pre- and postsynaptic 5-HT1A receptors: *in vivo* microdialysis and electrophysiological studies in the rat brain. [erratum appears in Neuropsychopharmacology 1997 Jan;16(1):91]. *Neuropsychopharmacology* **15**: 349–360.

Samama P, Cotecchia S, Costa T, Lefkowitz RJ (1993). A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model. *J Biol Chem* **268**: 4625–4636.

Sanchez C, Arnt J, Moltzen E (1996). Assesment of relative efficacies of 5-HT$_{1A}$ receptor ligands by means of *in vivo* animal models. *Eur J Pharmacol* **315**: 245–254.

Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J *et al* (2000). Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry* **57**: 174–180.

Schotte CK, Maes M, Cluydts R, De Doncker D, Cosyns P (1997). Construct validity of the Beck Depression Inventory in a depressive population. *J Affect Disord* **46**: 115–125.

Serrats J, Artigas F, Mengod G, Cortes R (2000). *Autoradiographic Detection of [^{35}S]GTPγS Binding in Rodent and Human Brain Reveals a Neutral Antagonist Action of (+/−)pindolol at Pre- and Post-synaptic 5-HT Receptors*. Society for Neuroscience: New Orleans. pp 120.

Sharp T, Bramwell SR, Graham-Smith DG (1989). 5-HT1 agonists reduce 5-hydroxytryptamine release in rat hippocampus *in vivo* as determined by brain microdialysis. *Br J Pharmacol* **96**: 283–290.

Sinton C, Fallon SL (1988). Electrophysiological evidence for a functional differentiation between subtypes of the 5-HT1 receptor. *Eur J Pharmacol* **157**: 173–181.

Spinks TJ, Jones T, Bailey DL, Townsend DW, Grootoonk S, Bloomfield PM *et al* (1992). Physical performance of a positron tomograph for brain imaging with retractable septa. *Phys Med Biol* **37**: 1637–1655.

Sprouse J, Braselton J, Reynolds L (2000). 5-HT$_{1A}$ agonist potential of pindolol: electrophysiologic studies in the dorsal raphe nucleus and hippocampus. *Biol Psychiatry* **47**: 1050–1055.

Sprouse JS, Aghajanian GK (1987). Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. *Synapse* **1**: 3–9.

Sprouse JS, Aghajanian GK (1988). Responses of hippocampal pyramidal cells to putative serotonin 5-HT1A and 5-HT1B agonists: a comparative study with dorsal raphe neurons. *Neuropharmacology* **27**: 707–715.

VanderMaelen CP, Matheson GK, Wilderman RC, Patterson LA (1986). Inhibition of serotonergic dorsal raphe neurons by systemic and ionophoretic administration of buspirone, a non-benzodiazepine anxiolytic drug. *Eur J Pharmacol* **129**: 123–130.

Verge D, Daval G, Marcinkiewicz M, Patey A, el Mestikawy S, Gozlan H *et al* (1986). Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J Neurosci* **6**: 3474–3482.

Whaley BS, Yuan N, Birnbaumer L, Clark RB, Barber R (1994). Differential expression of the beta-adrenergic receptor modifies agonist stimulation of adenylyl cyclase: a quantitative evaluation. *Mol Pharmacol* **45**: 481–489.

Yocca FD, Iben L, Meller E (1992). Lack of apparent receptor reserve at postsynaptic 5-hydroxytryptamine1A receptors negatively coupled to adenylyl cyclase activity in rat hippocampal membranes. *Mol Pharmacol* **41**: 1066–1072.