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β-blocker Binding to Human 5-HT_{1A} Receptors *in vivo* and *in vitro*: Implications for Antidepressant Therapy

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A novel strategy for improving the treatment of depressive illness is augmentation of antidepressants with a 5-$HT1_{1A}$ autoreceptor antagonist. However, trials using the 5-$HT1_{1A}$/β-blocker pindolol are proving inconsistent. We report how positron emission tomography (PET) and in vitro *autoradiography can inform trials of antidepressant augmentation. We show that in healthy volunteers,* in vivo, *pindolol (n = 10) and penbutolol (n = 4), but not tertatolol (n = 4) occupy the human 5-HT_{1A} receptors, at clinical doses. Pindolol, as well as the β-blockers penbutolol and tertatolol, has high affinity for human 5-HT_{1A} receptors in post-mortem brain slices (n = 4). Pindolol shows preference for 5-HT_{1A} autoreceptors versus the post-synaptic receptors both* in vitro *and* in vivo. *Our data reveal that pindolol doses used in antidepressant trials so far are suboptimal for significant occupancy at the 5-HT_{1A} autoreceptor. Penbutolol or higher doses of pindolol are candidates for testing as antidepressant augmenting regimes in future clinical trials.* ***[Neuropsychopharmacology 23:285–293, 2000]*** *© 2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc. All rights reserved.*

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SSRIs are increasingly used in the pharmacological treatment of depression. SSRIs specifically block 5-HT reuptake sites leading to an eventual increase of 5-HT in terminal synapses; an effect considered to be an important component of antidepressant action (Blier et al. 1990). SSRIs are better tolerated than less selective agents such as tricyclic antidepressants (TCAs) (Anderson 1997), however, their efficacy is no greater (Rudorfer and Potter 1989) (≅70% of patients respond), and at least 2-3 weeks of treatment is required for the appearance of meaningful therapeutic response (Paykel and Priest 1992).

Preclinical studies suggest the inhibition of 5-HT reuptake by SSRIs, does not lead to increased 5-HT levels in terminal synapses until an adaptive desensitization of inhibitory 5-HT_{1A} autoreceptors has occurred (Blier et al.

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1990; Chaput et al. 1986;Chaput et al. 1991; de Montigny et al. 1990; Hjorth and Auerbach 1994). These autoreceptors, located on 5-HT neuronal cell bodies in the raphe nuclei, inhibit 5-HT neurone firing, and hence 5-HT release in terminal synapses (VanderMaelen et al. 1986; Adell and Artigas 1991; Invernizzi et al. 1992; Sharp et al. 1989). Thus, indirect 5-HT_{1A} autoreceptor activation by SSRIs (via elevated 5-HT in the raphe nuclei) may impair the drug's therapeutic efficacy until desensitization has occurred. Theoretically, co-administration of 5-HT_{1A} autoreceptor antagonists (blocking the tonic inhibitory effect of 5-HT on the autoreceptor), together with SSRIs, may decrease the time needed to achieve clinical benefit and lead to a greater therapeutic effect (Hjorth 1993; Artigas 1993). In the absence of selective 5-HT_{1A} receptor antagonists for human use, therapeutic trials have been conducted with pindolol, a β-blocker with nanomolar affinity for the human 5-HT_{1A} receptor in transfected cells in culture (Newman-Tancredi et al. 1998). Controlled clinical trials have produced mixed results. Four studies demonstrated a beneficial effect of pindolol augmentation of SSRI treatment (Zanardi et al. 1997; Perez et al. 1997; Bordet et al. 1998; Maes et al. 1996), one study showed a mixed response (Tome et al. 1997), while three studies failed to demonstrate any antidepressant benefit (Moreno et al. 1997; Berman et al. 1997; Perez et al. 1999).

The equivocal results of clinical studies raise several questions about pindolol's proposed mechanism of action. Firstly it is not clear whether the dose of pindolol used clinically (typically 2.5 mg thrice daily (tds)), achieves a significant occupancy of 5-HT_{1A} receptors in the living human brain. Secondly, emergent evidence from preclinical studies indicates that pindolol, unlike certain other β-blockers, may have 5-HT_{1A} partial agonist properties (Hjorth and Carlsson 1986; Newman-Tancredi et al. 1998; Clifford et al. 1998;Gartside et al. 1999). Partial agonism could lead to an activation of 5-HT_{1A} autoreceptors with subsequent decrease in 5-HT release in limbic and cortical areas, opposite to the desired effect. And thirdly, a dose of pindolol which blocks the 5-HT_{1A} autoreceptor might be expected to also block the post-synaptic 5-HT_{1A} receptor, potentially counteracting any antidepressant effect. Post-synaptic 5-HT_{1A} receptors are located in cortical and limbic areas (Peroutka 1985; Pazos et al. 1988), where they mediate the effects of 5-HT released from the nerve terminals (Blier et al. 1990; Chaput et al. 1991; Invernizzi et al. 1991; Sinton and Fallon 1988).

Two other β-blockers, penbutolol and tertatolol, also display nanomolar affinity for the 5-HT_{1A} receptor in rodent brain (Prisco et al. 1993; Jolas et al. 1993; Hjorth and Sharp 1993). In rat microdialysis studies penbutolol and tertatolol reliably increase the levels of extracellular 5-HT, unlike pindolol in some studies (Gartside et al. 1999), when co-administered with an SSRI. They may therefore be more suitable than pindolol as 5-HT_{1A} autoreceptor antagonists for augmentation of SSRIs.

In light of the above, the aims of the present study were; to test the affinity of pindolol, penbutolol and tertatolol at the 5-HT_{1A} autoreceptor and post-synaptic receptor, in the postmortem human brain, and assess the ability of clinical doses of these drugs to occupy the human 5-HT_{1A} autoreceptor and post-synaptic receptor sites *in vivo.*

MATERIALS AND METHODS

Subjects

Eighteen healthy male volunteers (ages 27–54) were recruited by a newspaper advertisement. All subjects gave informed consent. The study was approved by the Imperial College School of Medicine Ethics Committee and the Administration of Radioactive Substances Advisory Committee. The volunteers were examined by a qualified psychiatrist (EAR) and a modified SCID for DSM IV was employed to exclude concurrent psychiatric illness. They had a full physical examination, and were not on any concurrent medication. All subjects underwent two open label [^{11}C]WAY-100635 PET scans with a median interval of eight days between the scans (range 4-258 days). The first was a baseline scan while the second was conducted following a single oral dose of one of the three β-blockers.

Pindolol scans were conducted two hours following a 5-mg (n = 3), 10-mg (n = 4), or 20-mg (n = 3), dose of (+/−)pindolol (Visken, Sandoz Pharmaceuticals). Penbutolol scans were conducted 3 hours following a 40-mg (n = 2), or an 80-mg (n = 2) dose of (−)penbutolol (Levotol, Schwarz Pharma). Tertatolol scans were conducted 90 minutes following a 5-mg (n = 2), or a 10-mg dose (n = 2) of (+/−)tertatolol (Artex, Servier). The times of the scan were chosen to coincide with the t_{max} of the drug in plasma (pindolol 1-3 hours, penbutolol 2-4 hours, tertatolol 1-2 hours).

PET Scan 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). [^{11}C]WAY-100635 was prepared at the Cyclotron Unit by ^{11}C-carboxylation of a Grignard reagent (McCarron et al. 1996). Venous blood samples were taken for estimation of plasma levels of the β-blocker at the time of injection of radioligand (t = 0 seconds).

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 (Lammertsma and Hume 1996; Gunn et al. 1998). The cerebellum was chosen as a

reference tissue because it has an extremely low number of 5-HT$_{1A}$ receptors (Hall et al. 1996). The reference tissue model allows the estimation of relative ligand delivery ($R_I = K_{1\ \text{REGION OF INTEREST}} / K_{1\ \text{REFERENCE REGION}}$) and 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)). The reference tissue model was applied at the voxel level, using a basis function implementation (Gunn et al. 1997, 1998), and parametric maps of BP and R_I were generated. Occupancy of the 5-HT$_{1A}$ receptor sites was inferred as a reduction of BP, and hence B_{AVAIL}, under the assumption that f_2 and K_D remain constant for the two scans.

We examined the midbrain raphe nuclei (RN), a small beaded structure consisting of serotonergic cell bodies with a high concentration of pre-synaptic 5-HT$_{1A}$ autoreceptors. The RN is undetectable on MRI but is well defined on a [^{11}C]WAY-100635 PET image. For this reason the ROI for the RN was defined manually on an integral image (20-90 minutes) for each individual PET scan. The resultant ROI was then applied onto the BP and R_I images to generate the BP and R_I values. These values for the RN were assumed to be representative of 5-HT$_{1A}$ autoreceptor values.

Cortical and limbic regions of interest (ROIs) were defined on an averaged MRI image in MNI space (Montreal Neurological Institute), to create a generic ROI map. A [^{11}C]WAY-100635 template (Meyer et al. 1999), also in MNI space, was warped onto each individual [^{11}C]WAY-100635 image, using SPM96 (Friston et al. 1991) normalization with 12 parameters. The transformation parameters were then applied to the ROI map, producing an individualized ROI map for each subject. The individualized ROI map was applied to BP and R_I images to obtain regional values for these parameters. This technique allowed us to examine 14 brain ROIs in temporal, parietal, prefrontal and cingulate cortex, which contain post-synaptic 5-HT$_{1A}$ receptors. A representative value for post-synaptic 5-HT$_{1A}$ receptors from the mean BP in all post-synaptic regions, weighted for the region size, was calculated. The individual cortical and limbic regions were also examined as independent regions to elicit regional variations in the post-synaptic 5-HT$_{1A}$ receptor occupancy.

A set of previously acquired test-retest data (analyzed as above) of 10 healthy volunteers (9 males and 1 female, median age 32, range 22–56, screened by a qualified psychiatrist to exclude psychiatric, or general medical illness) scanned on two occasions with a median interval of 56 days (range 14-489 days), was used as a control group in the subsequent statistical analysis. 5-HT$_{1A}$ receptor occupancy (as per Equation 1) was calculated and compared to the differences in BP between the two scans of the control group. The R_I data were analyzed in a similar manner to the BP data [Eq. (1)].

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

Autoradiography

Human brain tissue was obtained at autopsy from four subjects (two men and two women, age 67 ± 9 years, post-mortem delay = 24.8 ± 3.6 hours) with no reported neurological or psychiatric disorders. Sequential 14-μm cryostat sections from hippocampus and dorsal raphe nucleus were thaw-mounted on to gelatinized slides and stored at −20°C until use. Autoradiographic procedure was modified from Khawaja (1995). Brain sections were preincubated for 30 minutes in 50 mM Tris-HCl buffer (pH = 7.5), prior to incubation with or without drug for two hours in 50 mM Tris-HCl buffer containing 10 μM pargyline with 3 nM [^{3}H]WAY-100635 (specific activity 80 Ci/mmol, Wyeth Research UK Ltd). Non-specific binding was determined using 10 mM unlabeled 5-HT. Following incubation, sections were washed 2 X 2 minutes in ice-cold buffer, briefly dipped in deionized water at 4°C and air-dried. Autoradiograms were generated by opposing labelled tissues to tritiated microscales and [^{3}H]-sensitive film for six weeks (Hyperfilm, Amersham plc, UK). Autoradiograms were quantified using a computerized image analysis system (MCID system, Image Research, St. Catherine's, Canada).

Statistical Analysis

Occupancy results were analyzed by means of one way ANOVAs, for the autoreceptor and the post-synaptic regions, with dose (0, 5, 10 and 20 mg), as the between-group factor. *Post hoc* Dunnett t-tests were performed to assess the influence of dose on each region. Differential occupancy effects were analyzed by means of paired Students t-tests.

RESULTS

Pindolol Achieves Significant Occupancy of the 5-HT$_{1A}$ Receptor

Both the 5-HT$_{1A}$ autoreceptor and the post-synaptic receptor sites showed a dose-dependent increase in 5-HT$_{1A}$ receptor occupancy by pindolol (Figure 1 and Table 1). All post-synaptic regions showed similar levels of occupancy for a given dose, with a high correlation of occupancy between all post-synaptic regions (mean r = 0.93, range: 0.77–0.99, for any two regions). Further analysis was therefore performed on an average of the post-synaptic 5-HT$_{1A}$ receptor regions and the autoreceptor region (RN). There was a significant effect of pindolol on

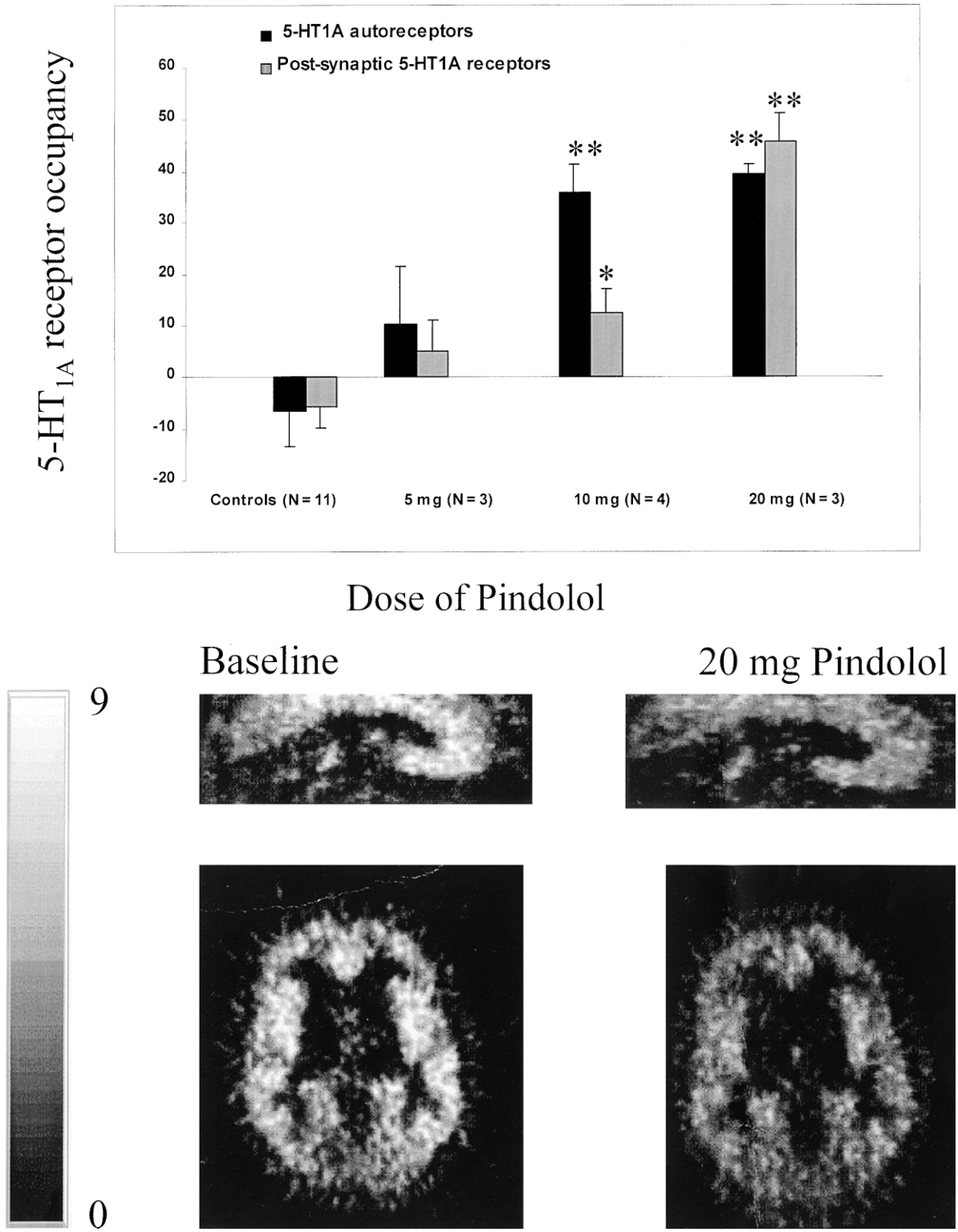

Figure 1. **(A)** 5-HT_{1A} receptor occupancy by pindolol in autoreceptor and averaged post-synaptic regions at different doses of pindolol. Bars are mean±SEM for each group. * $p < .05$, ** $p < .005$. **(B)** Transverse and sagittal BP images of a single subject before and after a 20-mg dose of pindolol.

5-HT$_{1A}$ receptor occupancy at both autoreceptors ($F(3,16) = 8.247$, $p < .005$), and post-synaptic receptors ($F(3,16) = 15.201$, $p < .001$). Post hoc *testing revealed that both the 10-mg and the 20-mg doses, but not the 5-mg dose, produced statistically significant occupancy of the 5-HT*$_{1A}$ receptor in both regions (Figure 1). There was no correlation between occupancy and duration of the between-scan interval.

Plasma pindolol levels were available for nine of the 10 subjects who received pindolol. Higher levels were associated with increased 5-HT$_{1A}$ receptor occupancy apart from one outlier (Table 1). We estimated the chronic dosing regime of pindolol required to achieve the plasma levels, which were associated with autoreceptor occupancy of at least 20 % in this study (plasma concentration that caused >20% occupancy was >12 ng/ml, Table 1). The equation, $\text{Dose} = C_{av}V_d\tau/1.44Ft_{1/2}$ (Rowland and Tozer 1980), gave an estimate of the dose of pindolol required to be given at an 8-hourly interval (τ), to achieve steady state plasma levels ($C\alpha\nu$) of 12 ng/ml. For pindolol: volume of distribution(V_d) = 1.6 L/kg (range 1.2-2.0), bioavailability(F) = 0.9, half-life ($t_{1/2}$) = 3.6 hours (range 2.5–4.0) (Dollery 1999). Calculating over the full range of values for V_d and $t_{1/2}$, the dose range for a 70 kg subject is 1.6–4.1 mg tds.

Pindolol had no significant effect on the relative delivery of radioligand to the region of interest, compared to the reference region (analysis of R_I values–Autoreceptor region, $F(3,16) = 0.491$, $p = .694$, Post-synaptic region, $F(3,16) = 1.166$, $p = .354$).

Pindolol Has a Differential Occupancy of Autoreceptor Compared to Post-synaptic Receptor Sites *in vivo*

There was a differential occupancy by pindolol of 5-HT$_{1A}$ autoreceptors compared to post-synaptic receptors at the 10-mg dose (37% vs. 13% respectively, paired t-test $t(3) = 19.224$, $P < 0.001$, Figure 1). This effect was not seen at other doses (20-mg 39% vs. 46%, $p = .245$; 5-mg 10% vs. 5%, $p = .576$; Control −7% vs. −6%, $p = .881$). We estimated the maintenance dose of pindolol required for plasma levels, which were associated with high differential occupancy of 5-HT$_{1A}$ receptors (12-18 ng/ml, Table 1). Using $\text{Dose} = C_{av}V_d\tau/1.44Ft_{1/2}$ as above, the dose range for a 70 kg patient is 1.6-6.2 mg tds.

Penbutolol but not Tertatolol Produces Consistent Occupancy of the 5-HT$_{1A}$ Receptor

Penbutolol at both the 40 mg and the 80 mg dose also occupied 5-HT$_{1A}$ receptors in both autoreceptor and post-synaptic receptor regions. At the doses tested (5 and 10 mg) tertatolol did not demonstrate consistent occupancy of the 5-HT$_{1A}$ receptor at either site, with only one subject at the higher dose, showing an effect (Table 2). We saw no differential 5-HT$_{1A}$ autoreceptor to post-synaptic receptor occupancy at either of the penbutolol doses examined.

Pindolol but not Penbutolol or Tertatolol Displays Differential Affinity for the 5-HT$_{1A}$ Autoreceptor Compared to the Post-synaptic Receptor *in vitro*

Pindolol, penbutolol and tertatolol displayed high affinity for 5-HT$_{1A}$ binding sites in postmortem human brain sections (Table 3). Given our *in vivo* results, and the suggestion by Romero (Romero et al. 1996) that pindolol preferentially affects autoreceptor versus post-synaptic 5-HT$_{1A}$ receptor function, we estimated the affinity of pindolol, penbutolol and tertatolol for the 5-HT$_{1A}$ autoreceptor compared to post-synaptic receptor. Pindolol, but not the other drugs displayed a difference in

Table 1. Individual subject 5-HT$_{1A}$ Receptor Occupancy by Pindolol, for the Autoreceptor (RN) and Post-Synaptic Receptor (Averaged Post-Synaptic Regions)

	Oral Dose	Autoreceptor Occupancy (%)	Post-synaptic Occupancy (%)	Differential Occupancy (%)	Pindolol plasma Concentration (ng/ml)
Subject 1	20 mg	39.3	41.9	−2.6	35.8
Subject 2	20 mg	42.7	56.6	−13.9	66.6
Subject 3	20 mg	35.8	38.7	−2.9	62.8
Subject 4	10 mg	23.7	2.2	21.5	11.8
Subject 5	10 mg	45.3	20.6	24.7	17.8
Subject 6	10 mg	38.1	14.3	23.7	14.2
Subject 7	10 mg	41.1	13.5	27.6	n.d.
Subject 8	5 mg	25.5	6.2	19.3	13.4
Subject 9	5 mg	−12.0	−5.5	−6.5	†68.7
Subject 10	5 mg	16.9	14.7	2.2	6.8

Pindolol Plasma Concentration Was Determined at the Time of Injection of the Radioligand (†This Value was Out of Keeping with the Oral Dose Given and the Occupancy Achieved). N.D. = Not determined.

Table 2. Individual Subject 5-HT_{1A} Receptor Occupancy By Penbutolol and Tertatolol for the Autoreceptor (RN) and Post-Synapti Receptor (Averaged Post-Synaptic Regions). Drug Plasma Concentration was Determined at the Time of Injection of the Radioligand

	Oral Dose	Autoreceptor Occupancy (%)	Post-synaptic Occupancy (%)	Differential Occupancy (%)	Plasma Concentration (ng/ml)
Penbutolol					
Subject 1	40 mg	29.5	39.0	−9.5	449
Subject 2	40 mg	30.2	27.3	2.9	229
Subject 3	80 mg	53.6	56.1	−2.5	4488
Subject 4	80 mg	26.0	26.5	−0.5	623
Tertatolol					
Subject 1	5 mg	3.1	4.3	−1.1	92.0
Subject 2	5 mg	−6.7	−9.6	2.9	45.7
Subject 3	10 mg	36.1	23.8	12.4	110.0
Subject 4	10 mg	0.9	−3.4	4.3	48.7

K_i at the autoreceptor compared to post-synaptic receptor. This difference was statistically significant (paired t-test, 1-tailed, $t(3) = 2.44$, $p = .047$). No significant differences in affinity for the autoreceptor versus post-synaptic receptor was seen for either penbutolol (paired t-test, 1-tailed, $t(3) = 0.145$, $p = .447$), or tertatolol (paired t-test, 1-tailed, $t(3) = -0.803$, $p = .240$).

DISCUSSION

Pindolol administered acutely to healthy subjects occupies the 5-HT_{1A} receptor in a dose dependent manner. A 20 mg dose produced substantial 5-HT_{1A} site occupancy (≅40%) at both the autoreceptor and the post-synaptic sites in limbic and cortical regions, consistent with previous pilot studies (Rabiner et al. 1998; Andree et al. 1999). Congruent with our findings, oral doses of 30 mg pindolol in man block both autoreceptor and post-synaptic functional responses to the 5-HT_{1A} receptor agonists, buspirone (Anderson and Cowen 1992) and flesinoxan (Seletti et al. 1995). We observed no consistent occupancy at either the 5-HT_{1A} autoreceptor or the post-synaptic receptor by 5 mg of pindolol. Pindolol levels > 12 ng/ml, in our study were associated with autoreceptor occupancy of > 20%. The mean plasma levels of pindolol achieved in the reported clinical studies of 2.5 mg tds of pindolol, 7.0 ng/ml (Artigas et al. 1997) and 9.9 ± 5.1 ng/ml (Perez et al. 1999), are in the lower range of levels achieved by our subjects and would be expected to produce low and/or variable occupancy of the 5-HT_{1A} autoreceptor (Table 1). A dose at the lower end of the effective range for occupancy may explain the variable results of controlled clinical trials. Our data, albeit in a small sample, and calculations suggest that a regimen of 5 mg tds may be required to produce consistent occupancy of the 5-HT_{1A} autoreceptor of about 20%. If a higher occupancy is needed to produce a clinically significant effect, then an even higher dose of pindolol would be required. Interestingly a similar conclusion has been reached in an independent study (M.Laruelle, personal communication).

The differential occupancy at 10 mg suggests that pindolol may have a higher affinity for the 5-HT_{1A} autoreceptor compared to the post-synaptic receptor. Although the number of subjects demonstrating this effect

Table 3. Affinity (K_I) of Pindolol, Penbutolol and Tertatolol for 5-HT_{1A} Receptors in the CA1 Region of the Hippocampus (CA1), and the Dorsal Raphe Nucleus (DRN), in Post-Mortem Human Brain

	Pindolol (nM)		Penbutolol (nM)		Tertatolol (nM)	
	CA1	DRN	CA1	DRN	CA1	DRN
Subject 1	18.6	6.5	15.6	16.9	22.8	18.7
Subject 2	13.0	8.0	11.8	9.7	11.2	21.1
Subject 3	13.7	11.7	10.9	13.2	26.7	20.5
Subject 4	12.1	9.2	10.0	7.8	12.2	32.9
Mean ± SD	14.4 ± 2.9	8.9 ± 2.2	12.1 ± 2.5	11.9 ± 4.0	18.2 ± 7.7	23.3 ± 6.5

is small, a similar differential occupancy was demonstrated in an independent study at another centre (M.Laruelle, personal communication). Laruelle and colleagues found differential occupancy also at the higher doses of pindolol, while we found no differential occupancy at the 20 mg dose. These findings are supported by our autoradiographic data, which demonstrates a slightly lower K_i for pindolol in the dorsal raphe nucleus, compared to the CA1 region of the hippocampus, although a recent study failed to show this difference in a similar sized sample (Raurich et al. 1999). The methods used in both studies were very similar. Therefore the discrepancy in results may be due to the small number of subjects in both studies (n = 4). Data from a larger number of postmortem brains are required to confirm or refute our *in vitro* results. An alternative explanation of the differential occupancy at autoreceptors and post-synaptic receptors may be a difference of microenvironment, or a difference in the proportion of G-protein coupled/G-protein uncoupled receptors between the raphe and cortical regions. Interestingly, a 2-fold higher affinity of pindolol for the 5-HT$_{1A}$ autoreceptor, compared to post-synaptic receptor, was reported in a recent rat PET study also using [^{11}C]WAY-100635 (Gunn et al. 1999; Hirani et al. 1999). Interestingly, we did not find a differential occupancy at the 20 mg dose, although only three subjects were studied. In contrast with our study, Laruelle et al report a differential occupancy at the higher doses (M. Laruelle, personal communication).

Differential occupancy may be of clinical importance. A dose of pindolol, which is able to preferentially antagonize 5-HT$_{1A}$ autoreceptors, without blocking post-synaptic sites, would facilitate SSRI induced release of 5-HT into the synaptic cleft in cortical and limbic areas, without blocking post-synaptic 5-HT$_{1A}$ receptors that mediate the effects of 5-HT. The standard dose of pindolol used in clinical trials, 2.5 mg tds, lies in the lower end of the range suggested by our calculations to lead to high differential occupancy (1.6-6.2 mg tds).

In our small sample penbutolol, but not tertatolol, in clinically approved doses, achieved consistent occupancy of the 5-HT$_{1A}$ receptor in humans *in vivo* despite their similar affinities *in vitro.* The lack of consistent occupancy of 5-HT$_{1A}$ receptors by tertatolol may be due to the low brain penetration by tertatolol (Campbell et al. 1986). The only subject showing appreciable 5-HT$_{1A}$ receptor occupancy had the highest plasma level of tertatolol. Our occupancy data suggest penbutolol may be a useful alternative 5-HT$_{1A}$ antagonist for further clinical investigation, especially as it has been shown to augment 5-HT release when combined with an SSRI (Gartside et al. 1999). However, doses of penbutolol which antagonize the 5-HT$_{1A}$ autoreceptor are also likely to block the post-synaptic receptor, as there was no differential occupancy at the autoreceptor and K_i were similar in the dorsal raphe nucleus and hippocampus on autoradiography (Table 3).

CONCLUSIONS

Our *in vitro* results demonstrate that all three drugs have a high affinity for the 5-HT$_{1A}$ receptor, with no pronounced regional selectivity, although pindolol displays a modest difference in affinity between the autoreceptor and post-synaptic receptor. Our *in vivo* study supports the theory that 5-HT$_{1A}$ autoreceptor site is an important target in the central action of pindolol. A dose higher than used currently will be required, if the aim of pindolol administration is to achieve consistent autoreceptor occupancy. We observed a differential occupancy of autoreceptor compared to post-synaptic receptor sites at the 10-mg dose of pindolol, which is consistent with the modest difference in affinity found in our *in vitro* study. Overall, our study demonstrates the utility of PET in directly testing novel sites of antidepressant drug action, and supports the notion that the 5-HT$_{1A}$ autoreceptor is a candidate site for antidepressant drug action.

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