Region-dependent effects of flibanserin and buspirone on adenylyl cyclase activity in the human brain

Donatella Marazziti[1], Lionella Palego[1], Annalisa Giromella[1], Maria Rosa Mazzoni[1], Franco Borsini[2], Norbert Mayer[2], Antonio Giuseppe Naccarato[3], Antonio Lucacchini[1] and Giovanni Battista Cassano[1]

[1] *Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologia, Università di Pisa, via Roma 67, 56100 Pisa, Italy*
[2] *Boehringer Ingelheim CNS Department, Ingelheim/Rhein, Germany*
[3] *Dipartimento di Oncologia, Università di Pisa, via Roma 67, 56100, Pisa, Italy*

Abstract

The mode of action of antidepressant drugs may be related to mechanisms of receptor adaptation, involving overall the serotonin 1A ($5\text{-}HT_{1A}$) receptor subtype. However, so far, the clinical effectiveness of selective compounds acting at this level has proved disappointing. This could be explained by the heterogeneity of $5\text{-}HT_{1A}$ receptors within the central nervous system. In animals, two $5\text{-}HT_{1A}$ agonists, flibanserin and buspirone, have shown different pharmacological properties, depending on the brain region. Since no evidence supports this observation in humans, this study sought to investigate whether these two drugs exert different effects on $5\text{-}HT_{1A}$ receptor activation in three different human brain areas: the prefrontal cortex, hippocampus and raphe nuclei. $5\text{-}HT_{1A}$-mediated inhibition of forskolin-stimulated adenylyl cyclase (AC) was taken as an index of $5\text{-}HT_{1A}$ receptor activation. Flibanserin significantly reduced the activity of AC post-synaptically, i.e. in the prefrontal cortex [EC_{50} (mean $\pm$ s.e.m.), 28 ± 10.2 nM; E_{max}, $18 \pm 2.3\%$] and in the hippocampus (EC_{50}, 3.5 ± 3.1 nM; E_{max}, $20 \pm 4.0\%$), but had no effect in the raphe nuclei, i.e. at pre-synaptic level. Vice versa, buspirone was only slightly but significantly effective in the raphe (EC_{50}, 3.0 ± 2.8 nM; E_{max}, $12 \pm 1.9\%$). Agonist effects were sensitive to the $5\text{-}HT_{1A}$ antagonists WAY-100135 and pindobind $5\text{-}HT_{1A}$ in the cortex and raphe nuclei, whereas buspirone antagonized flibanserin in the hippocampus. These findings suggest a region-related action of flibanserin and buspirone on forskolin-stimulated AC activity in human brain.

Introduction

In the search for new antidepressant compounds with a short latency of action, the serotonin (5-HT) receptor subtypes are the most widely investigated and, in particular, $5\text{-}HT_{1A}$ receptors, which have been found to play a key role in central serotonergic transmission (Lerer et al., 1999; Schreiber and De Vry, 1993). Several lines of evidence support the role of this receptor subtype in depression: blunted physiological responses to $5\text{-}HT_{1A}$ receptor agonists in vivo as well as the in-vitro changes in $5\text{-}HT_{1A}$ receptor binding in post-mortem brain tissues from depressed patients (Arango et al., 1995; Cowen et al., 1994; Lesch et al., 1992; Meltzer and Maes, 1995; Shapira et al., 2000; Stockmeier et al., 1998). Further, a widespread reduction of $5\text{-}HT_{1A}$ receptors labelled by the selective antagonist [^{11}C]WAY-100635 has been found in the living brain of depressed patients by positron emission tomographic scans (Drevets et al., 1999; Sargent et al., 2000). The use of pindolol, a $5\text{-}HT_{1A}$ pre-synaptic antagonist that leads to a direct blockade of $5\text{-}HT_{1A}$ auto-receptors, has produced a faster onset of action during combination treatment with SSRIs (Artigas et al., 1996; Marangell, 2000; Zanardi et al., 1997). In addition, selective agonists on post-synaptic $5\text{-}HT_{1A}$ receptors have been proposed as potential fast-acting antidepressant drugs (Blier and De Montigny, 1994). However, the clinical efficacy of $5\text{-}HT_{1A}$ receptor agonists is controversial. Buspirone and related azapirones, proposed as both anxiolytic and antidepressant agents on the basis of their direct activation of rodent $5\text{-}HT_{1A}$ receptors, have also shown a delayed therapeutic activity (Heiser and

Address for correspondence: Dr D. Marazziti, Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologia, via Roma 67, 56100 Pisa, Italy.
Tel.: +39-050-835412   *Fax*: +39-050-21581
E-mail: dmarazzi@psico.med.unipi.it

Wilcox, 1998). This could be explained in the light of the recently suggested heterogeneity of 5-HT$_{1A}$ receptors.

In animals, it has been observed that compounds acting on 5-HT$_{1A}$ receptors can exhibit different agonist/antagonist characteristics (Borsini, 1998; Borsini et al., 1995a,b; De Vry, 1995, for review; El Mansari and Blier, 1997). No pharmacological evidence regarding heterogeneity of 5-HT$_{1A}$ receptors is available for humans. Therefore, our study explored whether 5-HT$_{1A}$ receptors could display a pharmacological and functional heterogeneity in the human central nervous system (CNS). To achieve this aim, the effects of 5-HT$_{1A}$ receptor activation were investigated in post-synaptic (prefrontal cortex and hippocampus) and pre-synaptic (raphe nuclei) regions of post-mortem human brains, using buspirone and flibanserin, two 5-HT$_{1A}$ receptor agonists displaying different pharmacological characteristics in animals (Borsini et al., 1995a,b; 1998). Agonist-mediated inhibition of forskolin-stimulated adenylyl cyclase (AC) activity was taken as a marker of receptor activation, since 5-HT$_{1A}$ receptor coupling to G proteins has been found to be resistant to post-mortem degradation (Elliott and Reynolds, 1999; O'Neill et al., 1991; Palego et al., 1997, 1999). The effects of flibanserin and buspirone were further characterized by means of selective 5-HT$_{1A}$ antagonists. Before performing the experiments on AC, we assessed the affinity of the various compounds for human 5-HT$_{1A}$ receptors using the radioligand [^{3}H]8-hydroxy-2(di-*N*-propylamino) tetraline ([^{3}H]8-OH-DPAT) in the brain regions under investigation.

Method

Human brain tissues

Brains from 20 subjects (11 men and 9 women, aged between 53 and 88 yr, mean$\pm$s.D., 70 ± 9.4 yr) were collected during autopsy sessions, the post-mortem delay ranging from 16 to 65 h (mean$\pm$s.D., 29 ± 12 h). All subjects had died from causes not involving the CNS, either primarily or secondarily, and whose histories excluded any evidence of psychiatric illness or treatment with psychotropic drugs at least until just prior to their death. It was not possible to check whether the subjects had taken natural agents, e.g. St. John's Wort. Causes of death were heart failure ($n = 14$), respiratory failure ($n = 5$) or lung cancer ($n = 1$). Prefrontal cortex (Brodmann's areas: A9 and A10), hippocampus and raphe nuclei at ponto-mesencephalic level were identified and collected by pathologists. Microscopic examination was routinely carried out by pathologists to exclude the presence of brain atrophy or other alterations in all subjects. Samples were then rapidly cooled at 4 °C and immediately processed for AC assays. For [^{3}H]8-OH-DPAT binding

studies, tissue samples were first frozen in liquid nitrogen and then stored at -80 °C until assay, which was performed within 2 wk.

[^{3}H]8-OH-DPAT binding assay

The binding of [^{3}H]8-OH-DPAT (Amersham, UK, specific activity, 222 Ci/mmol) was carried out in order to measure the K_I (inhibition constant, nM) values of the tested 5-HT$_{1A}$ receptor compounds: some of them (flibanserin, WAY-100135) have never been evaluated in human brain tissues. [^{3}H]8-OH-DPAT saturation experiments and competition-binding studies were performed as described previously, by incubating membrane homogenates for 15 min at 37 °C (Marazziti et al., 1994).

AC activity

Partially purified membrane fractions from individual brain samples were prepared as follows: tissue blocks were homogenized by hand (20–30 strokes) in a glass potter Teflon homogenizer in 50 vols. (w/v) of a 10 mM Hepes–NaOH buffer (Hepes buffer), pH 7.4, containing 0.32 M sucrose, and centrifuged twice (1000 g for 10 min and 13 500 g for 20 min at 4 °C). The last pellet was re-suspended in 50 vols. (w/v) of Hepes buffer without sucrose and centrifuged at 35 000 g for 20 min at 4 °C. The final pellet was suspended in the same volume of Hepes buffer and incubated for 15 min at 24 °C. AC activity was measured in a reaction mixture of 50 mM Hepes buffer, 0.5 mM ATP, 2 mM MgCl$_2$, 1 mM cyclic AMP (cAMP), 0.5 mM isobutyl-methyl-xantine (IBMX), 50 U/ml creatine phosphokinase and myokinase, 5 mM creatine phosphate, 0.04 mM GTP and 1-2 μCi [α-^{32}P]-ATP, corresponding to $2–4 \times 10^6$ cpm per tube (Amersham, UK; specific activity, 30 Ci/mmol), both with and without (basal activity) 100 μM forskolin. The final volume of reaction mixture was 150 μl, composed of 50 μl of membranes ($\cong$ 30 μg protein/sample), 50 μl of the compound's solutions, consisting of agonists: flibanserin (0.01 nM–10 μM, Boehringer Ingelheim, Milan, Italy), buspirone (0.01 nM–10 μM, RBI, Natick, USA), 5-HT (0.01 nM–10 μM, Sigma–Aldrich, Milan, Italy), and antagonists: ($\pm$)-WAY-100135 (50–900 nM, Boehringer Ingelheim), pindobind 5-HT$_{1A}$ (12.5–25 nM, RBI), as well as 50 μl of the reaction mixture. The reaction was started by adding membranes and carried out by incubating samples for 15 min at 24 °C. Proteins were evaluated according to Peterson's method (Peterson, 1977), using bovine serum albumin as the standard. The reaction was stopped by placing samples in an ice bath and adding an ice-cold solution of 120 mM zinc acetate and 144 mM Na$_2$CO$_3$, as described by Johnson et al. (1994). The stop

Table 1. Affinity constants [K_I, (nM)] of displacement of [^{3}H]8-OH-DPAT binding

	Prefrontal cortex	Hippocampus	Raphe nuclei
Flibanserin	16.2 ± 1.5	15.0 ± 1.1	42.3 ± 5.4
Buspirone	62.5 ± 2.0	68.7 ± 2.3	98.3 ± 6.2
5-HT	7.4 ± 0.9	6.8 ± 0.8	$(-)$
$(\pm)$-WAY-100135	653 ± 65	$(-)$	796 ± 78

Values are means $\pm$ s.e.m. of five (flibanserin, buspirone and 5-HT in the prefrontal cortex and hippocampus) or three (flibanserin, buspirone, 5-HT and WAY-100135 in the raphe, and WAY-100135 in the prefrontal cortex) individual brain samples, each performed in triplicate. $(-)$, Not performed.

solution of zinc acetate contained 10 000–15 000 cpm/sample of [^{3}H]cAMP (Amersham, UK; specific activity, 27 Ci/mmol) in order to monitor column recovery. The formation of ZnCO$_3$ into assay tubes caused the precipitation of residual ATP through a centrifugation step at 2700 **g** for 8 min. Supernatants containing both [α-^{32}P]-cAMP and [^{3}H]cAMP were then passed through double-step Dowex-Alumina chromatography.

Data analysis

Drug competition-binding data were analysed with the iterative curve-fitting computer programs EBDA-LIGAND.

Equilibrium-binding parameters in the three brain areas were represented as B_{max}, maximal binding capacity (fmol/mg protein) and K_d, dissociation constant (nM). For evaluating AC activity (pmol cAMP/mg protein) Salomon's equation (1979) was used. Concentration–response curves of buspirone, flibanserin and 5-HT on forskolin-activated AC were analysed using the GraphPad computer program (GraphPad Software Inc., San Diego, CA, USA) in order to estimate EC$_{50}$ (concentration of drug causing 50% inhibition of enzyme activity, nM) and maximal inhibition (efficacy, E_{max}, %) values. The variability of data in the hippocampus was higher than in the cortex and raphe, and thus variances were not homogeneous. Therefore, the Wilcoxon non-parametric test was used to evaluate differences between basal, forskolin-stimulated enzyme activity or agonist inhibition (as pmol/min . mg protein) in each brain area. A paired *t* test was instead used to evaluate agonist inhibition, by normalizing AC activity at the E_{max} agonist concentration to percent of forskolin stimulation (considered to be 100%). An unpaired *t* test was applied to test differences between the E_{max} values of agonist and the endogenous ligand (5-HT). Statistical significance was preset at $\alpha = 0.05$.

Correlations between basal, forskolin-stimulated AC activity, agonist effects, subject's age and post-mortem delay, were performed using Pearson's method. All analyses were performed using the GraphPad Software Inc.

Results

Saturation binding experiments (mean $\pm$ s.e.m. of three individual brain samples performed in duplicate) showed that the B_{max} (fmol/mg protein) of [^{3}H]8-OH-DPAT in the raphe nuclei was 38 ± 1 and the K_d (nM) was $7.7 + 0.26$. Scatchard analysis of the data revealed the existence of a single population of binding sites. These values are quite consistent with those previously obtained by our research group with [^{3}H]8-OH-DPAT in the prefrontal cortex ($B_{max} = 51 \pm 3.5$; $K_d = 4.8 \pm 0.6$) and in the hippocampus ($B_{max} = 69 \pm 4.6$; $K_d = 3.9 \pm 0.6$) (Marazziti et al., 1994). Table 1 presents the affinity values of flibanserin, buspirone, 5-HT and WAY-100135 obtained by competition studies of [^{3}H]8-OH-DPAT binding in the three brain regions under study. Computer-assisted analysis of competition data for all compounds gave deep and monophasic curves (Hill coefficient not significantly different to 1) for the displacement of [^{3}H] 8-OH-DPAT from 5-HT$_{1A}$ receptors in the cortex, hippocampus and raphe nuclei.

Results of AC experiments showed that the basal AC activity (pmol cAMP/mg protein) (mean $\pm$ s.e.m.) was 121 ± 18.8 in the prefrontal cortex ($n = 16$), 95 ± 20.8 in the hippocampus ($n = 16$) and 107.8 ± 18.2 in the raphe nuclei ($n = 18$). The incubation of membranes with forskolin increased adenylyl activity (pmol cAMP/mg protein) significantly ($p < 0.001$) in the three tested regions, up to 310 ± 51.5 in the prefrontal cortex, 211 ± 52 in the hippocampus and 241 ± 45 in the raphe, representing the 253, 227 and 250% of the basal values, respectively. As shown in Table 2, flibanserin, up to a concentration of 10 μM, had no effect on forskolin-stimulated AC activity in the raphe nuclei, while reducing it significantly in the prefrontal cortex (from 318 ± 86.4 to

Table 2. Inhibitory effects of flibanserin, buspirone and 5-HT on forskolin-stimulated AC activity in the human brain

	Flibanserin		Buspirone		5-HT	
	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)	EC_{50} (nM)	E_{max} (%)
Prefrontal cortex	28.5 ± 10.2	18.5 ± 2.3^{b}	$> 10\,000$	–	5.6 ± 1.6	12 ± 0.9^{b}
Hippocampus	3.5 ± 3.1	19.8 ± 4.0^{a}	$> 10\,000$	–	0.16 ± 0.04	6.9 ± 0.9^{a}
Raphe	$> 10\,000$	–	3.0 ± 2.8	11.7 ± 1.9	6.8 ± 1.7	13.7 ± 0.7

Values represent the mean $\pm$ s.e.m. of individual brain samples [prefrontal cortex: flibanserin ($n = 8$), buspirone ($n = 4$), 5-HT ($n = 4$); hippocampus: flibanserin ($n = 6$), buspirone ($n = 3$), 5-HT ($n = 4$); raphe: flibanserin ($n = 7$), buspirone ($n = 6$), 5-HT ($n = 4$)].

EC_{50} (nM), concentration of drug causing 50% inhibition of enzyme activity (drug potency).

E_{max} (%), maximal response (drug efficacy), expressed as percentage of maximal drug inhibition with respect to forskolin-stimulated AC activity (considered to be 100%).

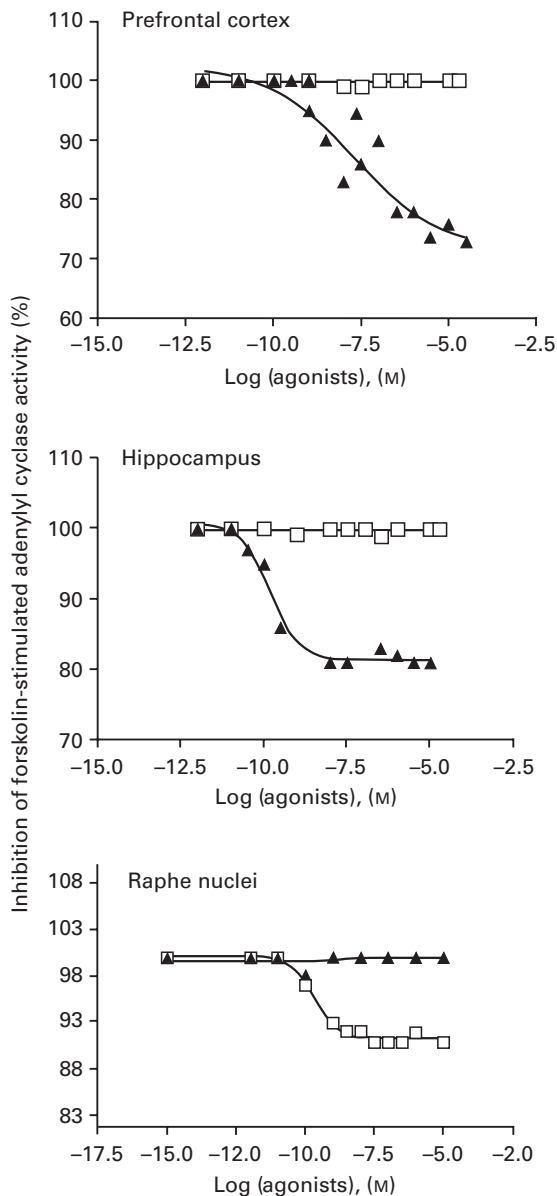[a] Significantly greater E_{max} of flibanserin than that of 5-HT in the hippocampus ($p = 0.035$).

[b] Nearly greater E_{max} of flibanserin than that of 5-HT in the cortex ($p = 0.09$).

252 ± 67.8, $p < 0.01$) and in the hippocampus (from 246 ± 137.2 to 202 ± 112.3, $p < 0.05$). Flibanserin displayed a significantly greater E_{max} than 5-HT in the hippocampus, while in the cortex this difference was not quite significant ($p = 0.09$). Buspirone was active only in the raphe, by inhibiting enzyme activity from 312 ± 106 to 282 ± 99 ($p < 0.05$). Despite buspirone E_{max} values were lower than those reported for 5-HT, this difference did not reach statistical significance. Normalized data for flibanserin and buspirone, plotted as percent of forskolin-stimulated AC activity (100%) in the three brain regions, are illustrated in Figure 1(a, b). A representative dose–response curve of flibanserin and buspirone obtained in the same area of the same subject is shown in Figure 2. The 5-HT$_{1A}$ antagonist $(\pm)$-WAY-100135 did not modify per se the forskolin-stimulated AC activity in the prefrontal cortex and raphe, but inhibited it in the hippocampus ($n = 2$), revealing an EC_{50} of 12.7 ± 12 nM and an E_{max} of 11 ± 3% (Figure 3). $(\pm)$-WAY-100135 competitively antagonized the effect of flibanserin in the prefrontal cortex and that of buspirone in the raphe (Figure 4). In fronto-cortical samples, flibanserin EC_{50} values shifted from approx. 1 nM to 9.5 or 205 nM, in the presence of 50 or 100 nM $(\pm)$-WAY-100135. Flibanserin EC_{50} values were > 10 μM (maximal concentration used) in presence of 500 or 900 nM $(\pm)$-WAY-100135. In the raphe, buspirone EC_{50} values shifted from 0.05 nM to 0.7, 2.5 or 123 nM, in the presence of 50, 100 or 500 nM $(\pm)$-WAY-100135. Flibanserin EC_{50} values were > 10 nM (maximal concentration used) in the presence of 900 mM $(\pm)$-WAY-100135.

Since $(\pm)$-WAY-100135 behaved as an agonist in the hippocampus, buspirone, which was inactive in this area, was used to antagonize flibanserin. Buspirone (50–
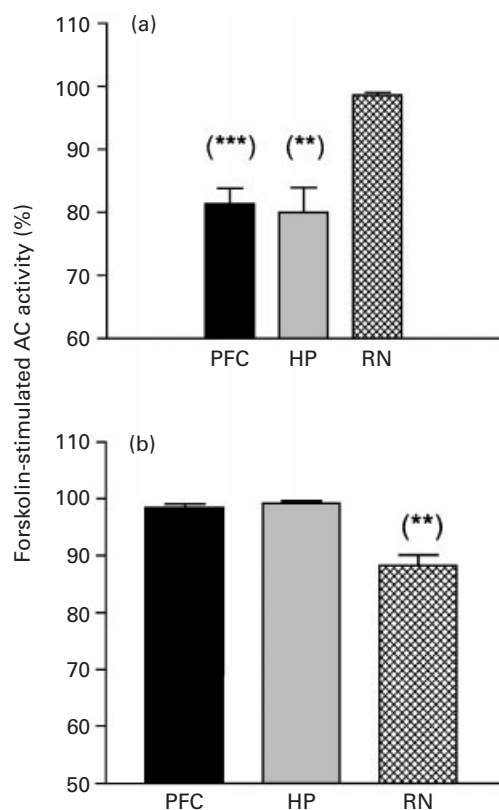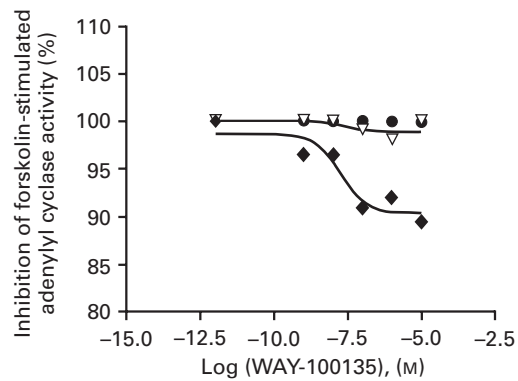

Figure 1. (a) Flibanserin- and (b) buspirone-mediated inhibition of forskolin-stimulated AC activity. PFC, prefrontal cortex; HP, hippocampus; RN, raphe nuclei. Data are plotted as percent of forskolin-stimulated AC activity (100%) at the E_{max} agonist concentration. (a) *** Significantly different from forskolin-stimulated AC activity (paired t test, $p = 0.0001$). ** Significantly different from forskolin-stimulated AC activity (paired t test, $p = 0.004$). (b) ** Significantly different from forskolin-stimulated AC activity (paired t test, $p = 0.016$).

Figure 3. Typical dose–response curves of ($\pm$)-WAY-100135 on forskolin-activated AC activity in the human prefrontal cortex ($\triangledown$), hippocampus ($\blacklozenge$) and raphe ($\bullet$).

(results not shown). In Figure 5, we observed that 25 nM pindobind 5-HT$_{1A}$ provoked a considerable increase of the EC$_{50}$ values of flibanserin in the cortex (two separate experiments; in Figure 5 a single experiment is shown); 12.5 and 25 nM pindobind 5-HT$_{1A}$ also caused a considerable increase in EC$_{50}$ values in the raphe for buspirone (a single experiment). Conversely, in the hippocampus, neither 12.5 nor 25 nM pindobind 5-HT$_{1A}$ was able to vary the flibanserin EC$_{50}$ values (a single experiment). No significant correlation between basal- and forskolin-stimulated AC velocities, agonist potency, subject's age or post-mortem delay was observed in the cortex and hippocampus. A significant positive correlation between post-mortem delay and buspirone efficacy was found in the raphe (results not shown).

Discussion

In the past decade, increasing evidence has been accumulated for the involvement of the 5-HT$_{1A}$ receptor system in depression. Thus, the study of the pharmacology of this receptor in the CNS seems relevant for elucidating the molecular substrates and mechanisms which may underlie depression.

The results of this study, assessed in post-mortem human brain, show that two 5-HT$_{1A}$ receptor agonists, flibanserin and buspirone, produced different effects on forskolin-stimulated AC activity depending on the brain region examined. Flibanserin was active in the prefrontal cortex and hippocampus while being inactive in the raphe. Conversely, buspirone was slightly but significantly active in the raphe but not in the cortex or hippocampus. The effects of flibanserin in the cortex and those of buspirone in the raphe nuclei were mediated by the activation of 5-HT$_{1A}$ receptors, as shown by the antagonism of WAY-100135 and pindobind 5-HT$_{1A}$. The mechanism of the action of flibanserin in reducing forskolin-stimulated AC

Figure 2. Representative dose–response curves of flibanserin ($\blacktriangle$) and buspirone ($\square$) on forskolin-stimulated AC activity carried out in the prefrontal cortex, hippocampus and raphe nuclei from the same autopsy subject.

150 nM) was able to shift the dose–response curve of flibanserin in the hippocampus rightwards (Figure 4). These studies of dose–response curve antagonism were done by using two samples for each area. Since four more samples were left, we used another 5-HT$_{1A}$ antagonist (pindobind 5-HT$_{1A}$) to evaluate the mechanism of action of flibanserin and buspirone in reducing forskolin-stimulated AC. We used pindobind 5-HT$_{1A}$ at 12.5 and 25 nM, since the affinity of this compound for 5-HT$_{1A}$ receptors was approx. 5 nM in the tested human brain regions
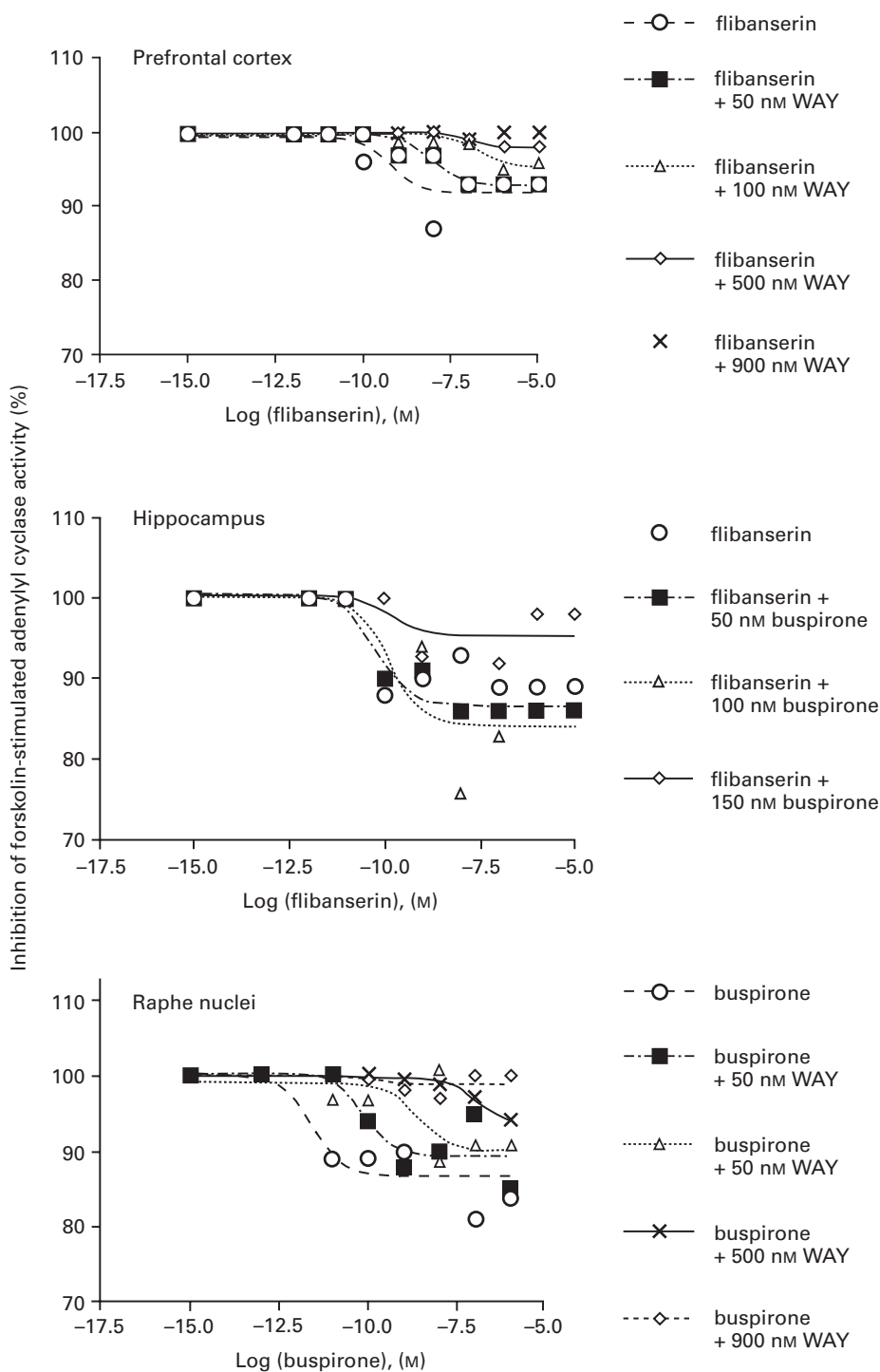
Figure 4. Effects of WAY-100135 (WAY) and buspirone on agonist-mediated inhibition of forskolin-stimulated AC activity in the post-mortem human brain. Points represent the mean of two separate experiments of two different subjects.

in the human hippocampus is more difficult to explain. In fact, in this area: (a) 5-HT displayed a poor efficacy in inhibiting AC activity; (b) WAY-100135, a specific 5-HT$_{1A}$ receptor antagonist, behaved surprisingly as an agonist; (c) buspirone competitively antagonized the

inhibition induced by flibanserin. Moreover, even if these experiments were carried out in a single sample for the hippocampus, up to 25 nM pindobind 5-HT$_{1A}$ was unable to induce a significant effect on flibanserin-mediated inhibition of AC. On the other hand, all the tested
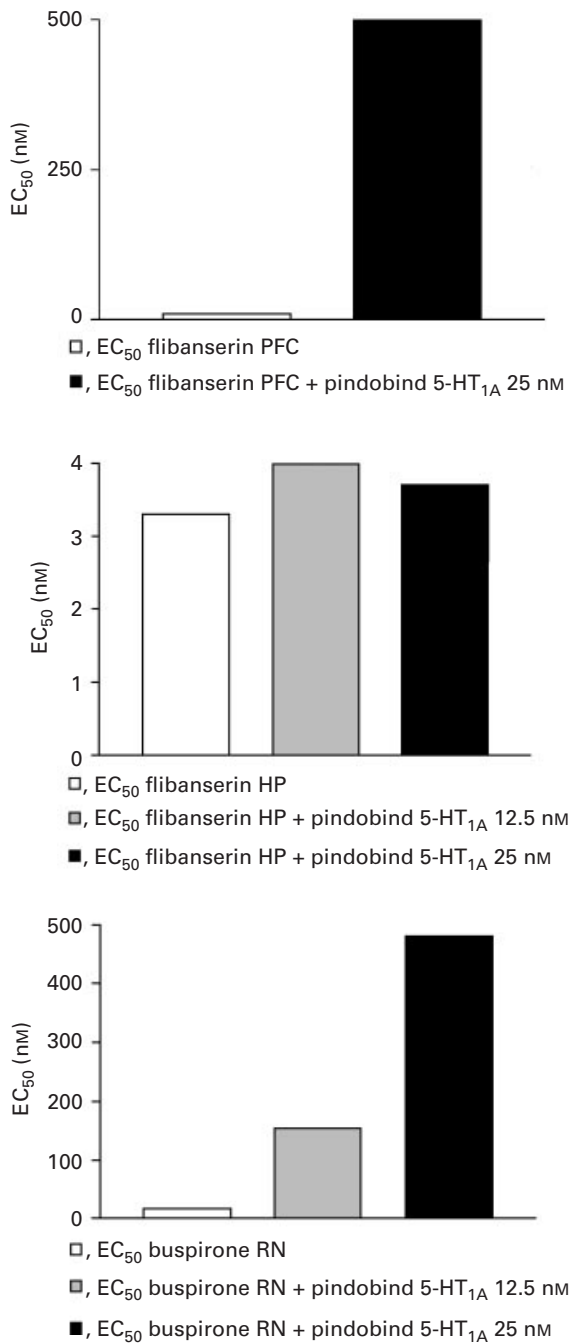
Figure 5. Effects of the selective antagonist pindobind 5-HT$_{1A}$ on agonist-mediated inhibition of AC activity in the brain regions under investigation (results are obtained from a single experiment). PFC, prefrontal cortex; HP, hippocampus; RN, raphe nuclei.

compounds were able to displace the 5-HT$_{1A}$ receptor binding by [^{3}H]8-OH-DPAT. Since we have previously observed that 8-OH-DPAT was equally active in inhibiting AC activity in the same human brain regions (Palego et al., 1999), flibanserin and buspirone seem to

produce a dissection in the pharmacology and function of central 5-HT$_{1A}$ receptors. The fact that buspirone acts as an antagonist in the human hippocampus seems surprising, since it has been found to act as a partial agonist in transfected cell lines (Newman-Tancredi et al., 1992). It seems likely that regional differences in drug action on 5-HT$_{1A}$ receptors can occur in humans. Recent lines of evidence in animals also support this view. Region-dependent changes in 5-HT$_{1A}$ receptor activated G-proteins have been observed in rat brain following chronic buspirone. In fact, this drug was able to desensitize 5-HT$_{1A}$ receptors in dorsal raphe without significant changes of cortical and hippocampal receptors (Sim-Selley et al., 2000). In addition, it has interestingly been observed that a region-dependent altered regulation of 5-HT$_{1A}$ receptor activity can occur in 5-HT transporter knock-out mice, similarly to that observed with chronic treatment with SSRIs, suggesting different adaptive response of this receptor subtype within the brain (La Cour et al., 2001). Flibanserin has been reported to bind also to 5-HT$_{2A}$ receptors, with a K_I of 133 nM in the rat (Borsini et al., 1998) and 115 nM in the human brain (D. Marazziti, unpublished observations). It has been also observed that 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors exert an opposite effect on neuronal membrane potential (Araneda and Andrade, 1991). Therefore, it cannot be excluded that the interaction with both subtypes may account for the regional differences of flibanserin reported here. Experiments with selective 5-HT$_{2A}$ receptor antagonists should be performed to exclude this possibility. The differences in 5-HT$_{1A}$ receptor density in these brain regions cannot account for the heterogeneous behaviour of flibanserin and buspirone, in fact buspirone was active in the raphe where the receptor density seems to be slightly lower than in the cortex and hippocampus. Interestingly, the 5-HT$_{1A}$ B_{max} value in the human raphe seems to be much lower than that reported in the rat raphe (Johnson et al., 1997). Comparison of buspirone and flibanserin E_{max} values with those of the 5-HT endogenous ligand revealed that buspirone had a lower E_{max} value than 5-HT, but this difference did not reach the statistical significance. Flibanserin displayed a significantly greater E_{max} value than 5-HT in the hippocampus, and to a lesser extent in the cortex (almost significant). Similar data have already been observed in the rat brain (Borsini et al., 1995a). The reported EC$_{50}$ mean values of flibanserin in the hippocampus and buspirone in the raphe (3.5 and 3.0 nM, respectively) are so different from affinity mean values obtained by these compounds in [^{3}H]8-OH-DPAT binding assays (15 and 98.3 nM, respectively). The same is true for 5-HT; in fact, in the hippocampus, 5-HT displayed a K_I value of 6.8 nM, but it reduced forskolin-stimulated AC with a potency value of 0.16 nM. Such discrepancies seem

to be consistent, since they have already been observed (Palego et al., 1999). Also the WAY-100135 effects appear to be discrepant from binding assay affinities. However, it should be considered that the experimental conditions of [^{3}H]8-OH-DPAT binding and AC assays are different. Different membrane preparations (homogenization procedures, presence or absence of Mg^{2+} or forskolin) or reaction buffers (Tris–HCl vs. Hepes–NaOH) and temperature (37 vs. 24 °C), must be taken into account. Moreover, in binding assay, agonist K_I values are calculated by competition with [^{3}H]8-OH-DPAT specific binding. Conversely, in AC assay, EC_{50} agonist values are estimated by the direct activation of 5-HT$_{1A}$ receptors in membrane suspensions. A possible explanation for agonist EC_{50}/K_I ratios that are lower than unity has been related to the concept of spare receptors (Yocca et al., 1992). Nevertheless, the fact that EC_{50} agonist values are lower than their K_I values is not conclusive for spare 5-HT$_{1A}$ receptors, as already observed for β-adrenoceptors (Johnson et al., 1979). Further, other authors reported no spare receptors for 5-HT$_{1A}$-mediated inhibition of AC in the rat hippocampus (Yocca et al., 1992). In addition, NaCl was not used in AC experiments: it is worth noting that the presence of NaCl has been reported to provoke a lower degree of agonist-mediated inhibition of forskolin-activated AC activity (Newman, 1994). Thus, the unusual agonism/antagonism profile observed in the human hippocampus may imply that 5-HT$_{1A}$ receptors in this area are somehow different, as has been suggested for rats (Nénoné et al., 1996). However, one cannot exclude the possibility that the discrepancy between EC_{50} and E_{max} values may depend on inter-subject variability, since agonism, antagonism and binding experiments were carried out in different autopsy subjects. It should also be taken into account that the mean age of autopsy subjects was 70 yr. Concerning the limits due to the use of post-mortem tissues, we observed a higher variability of data in the hippocampus than in the cortex and raphe, which cannot exclude a potential influence of post-mortem degradation on the present results. However, no significant correlation was found between agonist EC_{50}, E_{max}, age and post-mortem delay, except a significant positive correlation for buspirone in the raphe. Taken together these findings give initial support to the belief that the pharmacology of 5-HT$_{1A}$ receptors is also heterogeneous in humans, as reported in animals. The heterogeneity might derive from different RNA editing processes, different protein glycosylation or region-dependent coupling to G proteins (Anthony and Azmitia, 1997; Borsini et al., 1995a). In support of the latter hypothesis, some authors have recently suggested that the reserve of $G_{\alpha i}$ subtypes or AC isoforms can strongly modify the way 5-HT$_{1A}$ receptors activate the AC pathway (Albert et

al., 1999; Liu et al., 1999). This might also explain why 5-HT$_{1A}$ receptors are linked to AC in the human raphe (present results) but not in the rat raphe (Clarke et al., 1996), or why buspirone may reduce AC in animals (see Borsini, 1998), but not in the human hippocampus (present results). Finally, our results may have clinical implications. They would explain why buspirone is not apparently a fast-acting antidepressant and exerts a major role in the treatment of anxiety disorders, where 5-HT$_{1A}$ autoreceptors are supposed to be hypoactive, and 5-HT$_{1A}$ post-synaptic receptors to be hyperactive (Schreiber and De Vry, 1993). Our results would also support the hypothesis of flibanserin as a fast-acting antidepressant drug (Borsini et al., 1997; D'Aquila et al., 1997). In fact, to be fast-acting, pharmacological data have suggested that an antidepressant should have a selective agonistic effect on post-synaptic 5-HT$_{1A}$ receptors (Blier et al., 1997). Anyway, it must be pointed out that, at least in animals, it seems that 5-HT$_{1A}$ receptors may also activate the G_o protein–K$^+$ channel system (Clarke et al., 1996). Thus, the fact that buspirone is inactive in reducing AC in both the cortex and hippocampus while flibanserin, being inactive in the raphe, cannot rule out the possibility that these two compounds may have some activity on other 5-HT$_{1A}$-related second-messenger mechanisms in those brain regions. This can also explain the apparent discrepancy between our results and the observed in-vivo electro-physiological response of flibanserin in the rat dorsal raphe or buspirone in the rat hippocampus (Rueter et al., 1998; Tada et al., 1999). A possible extension of the present study would consist of investigating the degree of AC inhibition of flibanserin and buspirone on a G protein-dependent mechanism of AC activation, such as that mediated by PGE$_1$.

In conclusion, our study suggests that the human 5-HT$_{1A}$ receptors located in different brain regions, despite similar [^{3}H]OH-DPAT binding profiles, present a different functional pharmacology at the level of the inhibition of forskolin-stimulated AC activity.

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