Pharmacological diversity between native human 5-HT_{1B} and 5-HT_{1D} receptors sited on different neurons and involved in different functions

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1 The releases of [³H]5-hydroxytryptamine ([³H]5-HT) and of endogenous glutamic acid and their modulation through presynaptic h5-HT_{1B} autoreceptors and h5-HT_{1D} heteroreceptors have been investigated in synaptosomal preparations from fresh neocortical samples obtained from patients undergoing neurosurgery.

2 The inhibition by 5-HT of the K⁺ (15 mM)-evoked overflow of [³H]5-HT was antagonized by the 5-HT_{1B}/5-HT_{1D} receptor ligand GR 127935, which was ineffective on its own; this drug was previously found to behave as a full agonist at the h5-HT_{1D} heteroreceptor regulating glutamate release.

3 The recently proposed selective h5-HT_{1B} receptor ligand SB-224289 also prevented the effect of 5-HT at the autoreceptor, being inactive on its own; in contrast, SB-224289, at 1 μM, was unable to interact with the h5-HT_{1D} heteroreceptor.

4 The inhibitory effect of 5-HT on the K⁺-evoked overflow of glutamate was antagonized by the h5-HT_{1D} receptor ligand BRL-15572; added in the absence of 5-HT the compound was without effect. BRL-15572 (1 μM) was unable to modify the effect of 5-HT at the autoreceptor regulating [³H]5-HT release.

5 The selective 5-HT_{1A} receptor antagonist (+)-WAY 100135, previously found to be an agonist at the h5-HT_{1D} heteroreceptor regulating glutamate release, could not interact with the h5-HT_{1B} autoreceptor when added at 1 μM.

6 It is concluded that native h5-HT_{1B} and h5-HT_{1D} receptors exhibit a hitherto unexpected pharmacological diversity.

Keywords: Human cerebral cortex; 5-hydroxytryptamine release; glutamate release; 5-hydroxytryptamine-glutamate interaction; human native 5-hydroxytryptamine receptors; h5-HT_{1B} receptor; h5-HT_{1D} receptor

Abbreviations: BRL-15572, 1-phenyl-3-[4-(3-chlorophenyl)piperazin-1-yl] phenylpropan-2-ol dihydrochloride; GR 127935, N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxa-diazol-3-yl)[1,1-biphenyl]-4-carboxamide; SB-224289, 2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo[2,3-f]indole-3-spiro-4'-piperidine hydrochloride salt; (+)-WAY 100135, (S)-(+)-N-tert-butyl-3-[4-(2-methoxyphenyl) piperazin-1-yl]-2-phenylpropionamide dihydrochloride

Introduction

At least five 5-HT₁ receptor subtypes have been recognized, 5-HT_{1A}, 5-HT_{1B} (former nomenclature: 5-HT_{1Dβ}), 5-HT_{1D} (formerly termed 5-HT_{1Dα}), 5-HT_{1E} and 5-HT_{1F}. All subtypes are seven transmembrane, G-protein coupled receptors and all are thought to be negatively linked to adenylyl cyclase (Hoyer *et al.*, 1994; Martin & Humphrey, 1994).

Human 5-HT_{1B} (h5-HT_{1B}) and 5-HT_{1D} (h5-HT_{1D}) receptors have been reported to have relatively low (63%) overall amino acid homology; in apparent contrast, for a series of 19 structurally diverse compounds, the two cloned human receptors were found to be nearly indistinguishable in their binding affinities (Hartig *et al.*, 1992; Weinshank *et al.*, 1992).

Subsequently, however, the classical 5-HT₂ receptor antagonist ketanserin was proposed to permit discrimination between h5-HT_{1B} and h5-HT_{1D} receptors (Kaumann *et al.*, 1994; Pauwels & Colpaert, 1995; Zgombick *et al.*, 1995). More recently, using recombinant h5-HT_{1B} and h5-HT_{1D} receptors, the novel compounds SB-216641, SB-224289 and BRL-15572

have been found to discriminate the two subtypes. In binding studies on receptors expressed in CHO cells SB-216641 and SB-224289 had, respectively, 25 and 80 fold higher affinity for h5-HT_{1B} than h5-HT_{1D} receptors; conversely, BRL-15572 had 60 fold higher affinity for h5-HT_{1D} than h5-HT_{1B} receptors. In functional assays ([³⁵S]-GTPγS binding and cyclic AMP accumulation) with the above cloned receptor expression system, SB-216641 and BRL-15572 displayed agonist intrinsic activity at both h5-HT_{1B} and h5-HT_{1D} receptors (Price *et al.*, 1997), whereas SB-224289 behaved as an inverse agonist (Gaster *et al.*, 1998).

Functional studies with native human brain 5-HT receptors are relatively rare and have been almost exclusively focused on autoreceptors mediating feedback inhibition of 5-HT release (Schlicker *et al.*, 1985; 1997; Galzin *et al.*, 1992; Maura *et al.*, 1993; Raiteri, 1994; Fink *et al.*, 1995). These autoreceptors were initially classified as h5-HT_{1B} based exclusively on their insensitivity to ketanserin (Maura *et al.*, 1993; Fink *et al.*, 1995). More recently, Schlicker *et al.* (1997) found that SB-216641 is a preferential ligand at native h5-HT_{1B} receptors in human neocortex, in agreement with the above results with

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cloned receptors, although the compound was an antagonist at the autoreceptors devoid of any intrinsic activity.

A functional model of native brain h5-HT_{1D} receptor has recently been proposed: neocortical glutamatergic neurons in human cerebrocortex possess release-inhibiting heteroreceptors that, based on their sensitivity to sumatriptan and to ketanserin, were proposed to belong to the h5-HT_{1D} subtype (Maura *et al.*, 1998). Interestingly, GR 127935, a compound described as a potent and selective 5-HT_{1B}/5-HT_{1D} receptor antagonist (Skingle *et al.*, 1993; 1996), inhibited glutamate release similarly to sumatriptan, thus behaving as a full agonist at native h5-HT_{1D} receptors (Maura *et al.*, 1998). Recently the h5-HT_{1D} ligand BRL-15572 has been reported to behave as a potent antagonist at native h5-HT_{1D} heteroreceptors in human atrial appendages (Schlicker *et al.*, 1997).

To establish that native h5-HT_{1B} and h5-HT_{1D} receptors have distinct (and possibly opposing) function in the human brain and can be differentially acted upon by drugs is of great importance. Release-regulating 5-HT autoreceptors and 5-HT heteroreceptors regulating glutamate release have therefore been compared using various selective 5-HT receptor ligands and monitoring [³H]-5-hydroxytryptamine ([³H]-5-HT) and endogenous glutamate release from human neocortical synaptosomes. Based on the data obtained, at least six compounds appear to permit a clear-cut pharmacological discrimination between h5-HT_{1B} and h5-HT_{1D} native receptors.

Methods

Characteristics of human specimens

Samples of human cerebral cortex were obtained from 11 female and seven male patients (aged 19–70 years). The tissues had to be removed from patients undergoing neurosurgery either to remove deeply located tumours or to treat epilepsy resistant to antiepileptic drugs. Samples of frontal (6), temporal (9) and parietal (3) lobes were used. Since no significant differences between results obtained from the two groups of patients were observed, data have been pooled. Immediately after removal, the tissue was placed in a physiological salt solution kept at 2–4°C.

Release experiments

Crude synaptosomes were prepared within 2 h, at 2–4°C. The tissue was homogenized in 40 vol of 0.32 M sucrose, buffered at pH 7.4 with phosphate. The homogenate was centrifuged (5 min at 1000 × *g*), to remove nuclei and debris, and synaptosomes were isolated from the supernatant by centrifugation at 12,000 × *g* for 20 min. The synaptosomal pellet was then resuspended in a physiological medium having the following composition (mM): NaCl 125, KCl 3, MgSO₄ 1.2, CaCl₂ 1.2, NaH₂PO₄ 1, NaHCO₃ 22 and glucose 11 (aerated with 95% O₂ and 5% CO₂); pH 7.2–7.4. Protein was determined according to Bradford (1976).

Synaptosomes were incubated 15 min at 37°C in 95% O₂ and 5% CO₂, either with [³H]-5-HT (final concentration 0.08 μM) or without radioactive label (experiments of glutamate release). Experiments of both [³H]-5-HT and endogenous glutamate release were performed with the same tissue sample when a sufficient amount of tissue was available (six out of 18 patients). Identical aliquots of the synaptosomal suspension (0.6–1 mg of protein) were distributed in a set of parallel superfusion chambers maintained at 37°C (Raiteri *et*

al., 1974). Superfusion was then started with standard medium aerated with 95% O₂ and 5% CO₂, at a rate of 0.6 ml min⁻¹, and continued for 48 min. At min 39 synaptosomes were depolarized by a 90-s pulse of KCl (15 mM) replacing an equimolar concentration of NaCl. Fraction collection began at min 36, according to the following scheme: two 3-min samples (basal release) before and after one 6-min sample (basal release plus release evoked by high-K⁺). When 5-HT was used as an agonist in experiments of [³H]-5-HT release, citalopram (1 μM) (a 5-HT uptake inhibitor) was present from the beginning of superfusion. Agonists were added concomitantly with high K⁺, antagonists were present from 8 min before depolarization.

Amino acid determination

The amount of endogenous glutamate released or remaining in the synaptosomes after superfusion was measured by h.p.l.c. The glutamate content of synaptosomes was measured in the supernatant obtained after homogenization (Ultra Turrax, max speed, 20 s) in ice-cold distilled water and centrifugation at 20,000 × *g* for 10 min. The analytical method (Tonnaer *et al.*, 1983) involved precolumn derivatization with *o*-phthalaldehyde followed by separation on C₁₈ reverse phase chromatography column (Chrompack, 10 cm × 4.6 mm, 3 μm) and three solvent discontinuous gradient, from 23% methanol in acetate buffer 0.1 M pH 6 to 46% methanol in acetate buffer 0.1 M pH 5.8, in 22 min, at a rate of 0.9 ml min⁻¹.

Calculation

The amount of endogenous glutamate released in each fraction collected was expressed as pmol mg⁻¹ synaptosomal protein. The radioactivity released in each fraction was calculated as a percentage of the synaptosomal tritium at the onset of the respective collection period. The depolarization-evoked release (overflow) was estimated by subtracting basal release from total release. Depolarization-evoked overflow in the presence of drugs was calculated as percentage variation with respect to the control. Means ± s.e.mean of the given numbers of experiments are presented throughout. The data presented in the Figures were compared by two-tailed Student's *t*-test.

Materials

5-Hydroxytryptamine (5-HT) creatinine sulphate was purchased from Calbiochem, Los Angeles, CA, U.S.A. The following drugs were gifts: N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxa-diazol-3-yl)[1,1'-biphenyl]-4-carboxamide (GR 127935; Glaxo Group Research, Greenford, U.K.); (S)-(+)-N-tert-butyl-3-[4-(2-methoxyphenyl)piperazin-1-yl]-2-phenylpropionamide dihydrochloride ((+)-WAY 100135; Wyeth Research, Berkshire, U.K.); 2,3,6,7-tetrahydro-1'-methyl-5-[2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl) biphenyl-4-carbonyl] furo[2,3-f] indole-3-spiro-4'-piperidine hydrochloride salt (SB-224289) and 1-phenyl-3-[4-(3-chlorophenyl)piperazin-1-yl]phenylpropan-2-ol dihydrochloride (BRL-15572) (SmithKline Beecham Pharmaceuticals, Harlow, Essex, U.K.)

Results

Human cerebral cortex synaptosomes were labelled with [³H]-5-HT and depolarized in superfusion with KCl (15 mM). Basal tritium release was 1.10 ± 0.09% min⁻¹ and K⁺-evoked

overflow amounted to $3.50 \pm 0.15\%$ ($n=6$). The overflow of tritium elicited by K⁺ depolarization was inhibited by 40% when 5-HT (0.1 μM) was added to the superfusion medium, in keeping with previously reported results (Maura *et al.*, 1993).

The inhibitory effect of 5-HT was totally prevented by GR 127935 (1 μM) (Figure 1), a drug classified as a selective mixed 5-HT_{1B}/5-HT_{1D} receptor antagonist (Skingle *et al.*, 1993; 1996). GR 127935 (1 μM), added on its own, had no effect on the basal (not shown) or the K⁺-evoked tritium release from synaptosomes prelabelled with [³H]-5-HT (Figure 1).

SB-224289, a recently proposed selective h5-HT_{1B} receptor ligand (Roberts *et al.*, 1997; Gaster *et al.*, 1998), abolished the effect of 5-HT when present in the superfusion medium at 1 μM . At this concentration, SB-224289 did not modify, on its own, the basal (not shown) or the K⁺-evoked release of tritium (Figure 1).

The inhibition by 5-HT of the K⁺-evoked tritium overflow remained unchanged when BRL-15572 (1 μM), a recently proposed selective h5-HT_{1D} receptor ligand (Price *et al.*, 1997; Schlicker *et al.*, 1997), was added to the superfusion medium at 1 μM (Figure 1). At this concentration, the compound had little effect on its own.

The selective 5-HT_{1A} receptor antagonist (+)-WAY 100135 (1 μM ; Fletcher *et al.*, 1993) was also unable to prevent the inhibitory effect of 5-HT and, added alone at 1 μM , did not affect the basal release (not shown) or the K⁺-evoked overflow of tritium (Figure 1).

Figure 2 illustrates the results obtained in experiments in which human cerebral cortex synaptosomes were depolarized with KCl (15 mM) and the release of endogenous glutamate was monitored. Basal glutamate release before the onset of the K⁺ stimulation amounted to 290 ± 38 pmol mg⁻¹ protein ($n=6$); the K⁺-evoked glutamate overflow was 505 ± 43 pmol mg⁻¹ protein ($n=6$). The overflow of glutamate provoked by high-K⁺ had previously been found to be strongly dependent on the presence of Ca²⁺ ions in the superfusion medium (Maura *et al.*, 1998).

When 5-HT (0.1 μM) was added concomitantly with the depolarizing stimulus, the glutamate overflow elicited by K⁺ (15 mM) was inhibited by 40% (Figure 2), which is in keeping with the concentration-response curve of 5-HT obtained previously, under identical experimental conditions (Maura *et al.*, 1998).

The inhibitory effect of 5-HT (0.1 μM) on the K⁺-evoked glutamate overflow from human neocortical synaptosomes

Figure 2 Antagonism by BRL-15572 or SB-224289 of the inhibition by 5-HT of the K⁺-evoked glutamate overflow from human cerebrocortical synaptosomes. Data are expressed as per cent inhibition of the K⁺-evoked overflow of glutamate. The agonist was added concomitantly with high-K⁺; the antagonists 8 min before. For more technical details see Methods. Data are means $\pm$ s.e. mean of 3–4 independent experiments, each consisting of three replicate chambers for each condition. * $P < 0.005$ when compared to control K⁺-evoked overflow.

Figure 1 Antagonism by GR 127935, SB-224289, BRL-15572 or (+)-WAY 100135 of the inhibition by 5-HT of the K⁺-evoked [³H]-overflow from human cerebrocortical synaptosomes prelabelled with [³H]-5-HT. Data are expressed as per cent inhibition of the K⁺-evoked overflow of tritium. The agonist was added concomitantly with high-K⁺; the antagonists 8 min before. For more technical details see Methods. Data are means $\pm$ s.e. mean of 3–6 independent experiments, each consisting of three replicate chambers for each condition. * $P < 0.005$ when compared to control K⁺-evoked overflow.

was completely prevented by BRL-15572 (1 μ M). At this concentration, the compound alone had no effect on the basal release (not shown) or the K⁺-evoked overflow of glutamate (Figure 2). In contrast SB-224289 (1 μ M) had no significant effect alone or on the 5-HT inhibition of glutamate overflow elicited by depolarization (Figure 2).

To facilitate comparison between the pharmacology of native h5-HT_{1B} auto- and h5-HT_{1D} heteroreceptors, the present data and the results previously obtained with human cerebral cortex have been summarized in Table 1.

Discussion

In the human cerebral cortex, the 5-HT autoreceptor located on serotonergic terminals and which mediates inhibition of 5-HT release probably represents the most important function of the h5-HT_{1B} receptor subtype (Fink *et al.*, 1995). Recently, a sumatriptan- and ketanserin-sensitive 5-HT heteroreceptor mediating inhibition of glutamate release was found to exist on glutamatergic nerve terminals of human brain cortex and proposed to represent a functional model for the h5-HT_{1D} subtype (Maura *et al.*, 1998). The compounds listed in Table 1 are ligands at h5-HT_{1B}/h5-HT_{1D} receptors, based largely on studies with recombinant receptors and, more rarely, with native human CNS receptors. The analysis of these compounds in release experiments with superfused human neocortex synaptosomes has now been completed and the Table summarizes their classification as agonists or antagonists (or inactive molecules) at the native h5-HT_{1B} and h5-HT_{1D} receptors considered.

Sumatriptan, a h5-HT_{1B}/h5-HT_{1D} ligand, is an agonist at both auto- and heteroreceptors (Maura *et al.*, 1993; 1998). The drug also behaved as a h5-HT_{1B}/h5-HT_{1D} receptor agonist in experiments with slices of human cerebral cortex, where it was found to inhibit [³H]-GABA release (Feuerstein *et al.*, 1996).

Methiothepin has often been shown to be an antagonist at native human brain 5-HT_{1B}/5-HT_{1D} receptors. It blocked h5-HT_{1B} terminal autoreceptors both in slices (Schlicker *et al.*, 1985; Galzin *et al.*, 1992) and in synaptosomes (Maura *et al.*, 1993; Fink *et al.*, 1995). Methiothepin also antagonized 5-HT and sumatriptan at the h5-HT_{1D} heteroreceptors regulating glutamate release (Maura *et al.*, 1998).

Ketanserin was the first compound proposed to permit discrimination between h5-HT_{1B} and h5-HT_{1D} (Kaumann *et al.*, 1994; Pauwels & Colpaert, 1995). Ketanserin exhibited

marked selectivity (~ 70 fold) for the recombinant h5-HT_{1D} relative to the h5-HT_{1B} receptor (Zgombick *et al.*, 1995). Accordingly, we recently found that ketanserin antagonized the sumatriptan-induced inhibition of glutamate release, whereas the sumatriptan-sensitive human neocortex autoreceptor was ketanserin-insensitive (Maura *et al.*, 1993; Fink *et al.*, 1995). It has to be noted that the affinity and resulting selectivity of ketanserin for the 5-HT_{1D} receptor is species dependent: the drug is only 5 fold selective for the guinea-pig 5-HT_{1D} receptor relative to the guinea-pig 5-HT_{1B} subtype (Zgombick *et al.*, 1997).

We find here that GR 127935 is a terminal autoreceptor h5-HT_{1B} antagonist, devoid of intrinsic activity (Figure 1), whereas at the h5-HT_{1D} heteroreceptor regulating glutamate release, GR 127935 was recently reported to behave as a full agonist (Maura *et al.*, 1998). The compound is usually classified as a 'mixed 5-HT_{1B}/5-HT_{1D} receptor antagonist', based on functional data from experimental animals (Skingle *et al.*, 1993; 1996). However, GR 127935 has been tested in several works carried out with recombinant h5-HT_{1B} and h5-HT_{1D} receptors and the data obtained, although in part controversial, possibly due to the different properties of the transfection hosts and differences in receptor expression levels, show that GR 127935 can display intrinsic activity. GR 127935 was a full agonist in HeLa cells expressing h5-HT_{1D} and h5-HT_{1B} receptors (Walsh *et al.*, 1995), and NIH-3T3 fibroblast expressing h5-HT_{1D} receptors. It was a partial agonist in Y-1 adrenocortical cells expressing h5-HT_{1B} receptors (Zgombick *et al.*, 1996); whereas, similarly to what observed in our present work, GR 127935 was an antagonist at cloned h5-HT_{1B} receptors, in transfected CHO cells, while showing intrinsic agonist activity at the h5-HT_{1D} subtype (Pauwels & Colpaert, 1995; Pauwels *et al.*, 1997). Interestingly, GR-127935 showed partial agonist activity at recombinant h5-HT_{1D} receptors in a cell line where no receptor reserve existed (Watson *et al.*, 1996).

Metergoline is known as a high affinity ligand at 5-HT_{1B}/5-HT_{1D} receptors (Hamblin & Metcalf, 1991; Bruinvels *et al.*, 1992). Strong intrinsic activity was observed with the drug at cloned h5-HT_{1D} receptors, but not at the h5-HT_{1B} subtype (Pauwels *et al.*, 1996). In keeping with these results, we found metergoline unable to antagonize 5-HT at the h5-HT_{1D} heteroreceptor regulating glutamate release; instead, metergoline inhibited the K⁺-evoked overflow of glutamate (Maura *et al.*, 1998), thus behaving as a h5-HT_{1D} receptor agonist (Table 1). Conversely, at the concentration displaying maximal agonist activity at the h5-HT_{1D} heteroreceptor, metergoline blocked 5-HT at the autoreceptors regulating 5-HT release in the human neocortex (Galzin *et al.*, 1992; Maura *et al.*, 1993) and it should therefore be considered as a potent antagonist at the native h5-HT_{1B} subtype.

SB-224289 has been classified as a selective 5-HT_{1B} receptor antagonist (Roberts *et al.*, 1997; 1998; Gaster *et al.*, 1998). Accordingly, the compound blocked the effect of 5-HT on the terminal autoreceptor in human neocortex synaptosomes. Added to the superfusion medium in absence of exogenous 5-HT, SB-224289 had no effect on the K⁺-evoked overflow of [³H]-5-HT. In contrast, at the heteroreceptors regulating glutamate release SB-224289 was unable to prevent the action of 5-HT; moreover, in the absence of 5-HT, it did not inhibit the K⁺-evoked overflow of glutamate. Thus SB-224289 is a pure antagonist at the h5-HT_{1B} autoreceptor at concentrations which have no effect on the h5-HT_{1D} heteroreceptor.

BRL-15572 behaved as a pure antagonist at the h5-HT_{1D} heteroreceptor regulating glutamate release in human cere-

Table 1 Pharmacological profiles of the h5-HT_{1B} autoreceptor and of the h5-HT_{1D} heteroreceptor regulating glutamate release in human cerebral cortex

Drugs	h5-HT _{1B} autoreceptor	h5-HT _{1D} heteroreceptor
Sumatriptan	Agonist ^a	Agonist ^b
Methiothepin	Antagonist ^{a,c}	Antagonist ^b
Ketanserin	Inactive ^{a,c}	Antagonist ^b
GR 127935	Antagonist	Agonist ^{b*}
Metergoline	Antagonist ^{a,d}	Agonist ^{b*}
SB-224289	Antagonist	Inactive
BRL-15572	Inactive	Antagonist
(+)-WAY 100135	Inactive	Agonist ^{b*}

^aData from Maura *et al.* (1993); ^bMaura *et al.* (1998); ^cFink *et al.* (1995); ^dGalzin *et al.* (1992). *Full agonist; maximal effect at 1 μ M, as with 5-HT or sumatriptan (Maura *et al.*, 1998).

brocortex, while exhibiting little or no affinity for the h5-HT_{1B} autoreceptor. In a recent paper (Schlicker *et al.*, 1997), BRL-15572 was reported to block the effect of 5-HT on the electrically-evoked release of [³H]-noradrenaline from human atrial appendages, an effect mediated by presynaptic h5-HT_{1D} heteroreceptors on noradrenergic terminals (Molderings *et al.*, 1996).

The 5-HT_{1A} receptor antagonist (+)-WAY 100135 had previously been found to inhibit the K⁺-evoked overflow of glutamate from human neocortex synaptosomes. Actually, in experiments run in parallel with sumatriptan, the inhibition caused by (+)-WAY 100135 (1 μ M) did not differ significantly from that caused by sumatriptan (1 μ M), the maximally effective concentration of this drug (Maura *et al.*, 1998). Thus (+)-WAY 100135, similarly to GR 127935, metergoline and spiperone (Maura *et al.*, 1998), appears to be a full agonist at h5-HT_{1D} heteroreceptors regulating glutamate release. On the other hand, (+)-WAY 100135 (1 μ M) was unable to reduce the action of 5-HT on the autoreceptor; moreover, the compound had no effect on [³H]-5-HT release, in the absence of 5-HT, indicating poor, if any, affinity for the h5-HT_{1B} autoreceptor (Table 1). Our data are in good agreement with those recently reported by Davidson *et al.* (1997) showing that (+)-WAY 100135 had much higher affinity for recombinant h5-HT_{1D} than h5-HT_{1B} receptors and behaved as a partial agonist in [³⁵S]-GTP γ S binding.

To conclude, our functional studies of native h5-HT_{1B} and h5-HT_{1D} receptors in the cerebral cortex clearly show that the two receptors, originally reported to be pharmacologically indistinguishable (Weinshank *et al.*, 1992), exhibit in fact a strikingly different pharmacology. Oddly enough, the diversity between these two receptor subtypes, as it emerges from the data summarized in Table 1, appears much more convincing than for other receptor systems, for instance the muscarinic receptors, where subtypes have often been proposed on the basis of relatively modest quantitative differences between antagonist affinities.

The finding that compounds which, based on experiments with laboratory animals, are known as pure receptor

antagonists (GR 127935, metergoline, (+)-WAY 100135), exhibit high intrinsic activity at native h5-HT_{1D} heteroreceptors, whereas none of the antagonists tested show intrinsic activity at h5-HT_{1B} autoreceptors, deserves some comments. As previously mentioned, intrinsic agonist activity is sometimes displayed in recombinant systems by otherwise pure receptor antagonists due to excessive receptor expression and consequent receptor reserve (Hoyer & Boddeke, 1993). It is usually thought that these drugs would be silent antagonists in native tissues, unless a large receptor reserve exists. If this is true, our data might reflect the existence of a high reserve of h5-HT_{1D} heteroreceptors, but not of h5-HT_{1B} autoreceptors, in the human cerebral cortex. In any case, considering the variability of the results obtained with cell systems expressing recombinant receptors, but also the species variations in ligand affinities previously observed (see Zgombick *et al.*, 1997), it is evident that functional studies with native human receptors remain of unique importance.

The pharmacological diversity of human 5-HT_{1B} and 5-HT_{1D} receptors augurs well for the potential development of receptor-selective drugs. In particular, the clear differences existing between release-regulating h5-HT_{1B} terminal autoreceptors and h5-HT_{1D} glutamate terminal heteroreceptors in human brain opens the possibility to develop drugs that are selective autoreceptor antagonists or heteroreceptor agonists, or both, and which may represent novel therapeutic agents potentially useful in conditions characterized by defective serotonergic transmission or/and excessive glutamatergic transmission, including depression, epilepsy and neurodegenerative diseases.

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