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5-Hydroxytryptamine (5-HT)$_4$ receptors in *post mortem* human brain tissue: distribution, pharmacology and effects of neurodegenerative diseases

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1 The distribution, pharmacology and effects of neurodegenerative diseases on 5-HT$_4$ receptors in human brain have been characterized *in vitro*.

2 The 5-HT$_4$ receptor in *post mortem* human brain tissue was specifically labelled with [^{3}H]-GR 113808. In human putamen, this ligand labelled a homogeneous population of sites, with an apparent affinity ($-\log K_d$) of 10.1 and a density (B_{max}) of 5.73 fmol mg^{-1} tissue. The pharmacology of this site was characterized by use of a series of displacing ligands, and the following rank order of apparent affinities (with mean ± s.d. $-\log K_i$ values in parentheses) was generated: GR113808 (10.05 ± 0.04)> SDZ 205,557 (8.65 ± 0.08)> DAU 6285 (7.95 ± 0.04)> BIMU-1 (7.81 ± 0.06)> DAU 6215 (7.42 ± 0.23)> tropisetron (7.39 ± 0.23)> 5-HT (7.32 ± 1.00)> BIMU-8 (7.25 ± 0.04)> (R)-zacopride (5.82 ± 0.04). The Hill coefficients were not significantly different from unity, consistent with an interaction at a single site. A comparison of the affinities of these compounds with those obtained from guinea-pig striatum indicated no evidence of species differences.

3 The regional distribution of 5-HT$_4$ receptors was assessed by determining the density of binding sites for [^{3}H]-GR 113808. The distribution were as follows (with mean ± s.d. B_{max} values, fmol mg^{-1} tissue, in parentheses): caudate nucleus (8.7 ± 1.5), lateral pallidum (8.6 ± 5.5), putamen (5.7 ± 3.0), medial pallidum (3.8 ± 0.9), temporal cortex (2.6 ± 0.6), hippocampus (2.4 ± 0.8), amygdala (2.3 ± 1.1), frontal cortex (1.7 ± 0.5), cerebellar cortex (< 1.0). In these studies, the affinities of GR 113808 were not significantly different.

4 The density of 5-HT$_4$ receptors selected from regions of *post mortem* brains of patients with Parkinson's disease, Huntington's disease and Alzheimer's disease were compared to age-matched controls. In Parkinson's disease, there was no significant difference between control or patient values (mean ± s.d. B_{max} values, fmol mg^{-1} tissue; putamen, control 4.74 ± 0.07, patient 5.86 ± 1.48; substantia nigra, control 4.21 ± 2.56, patient 5.57 ± 0.10). In Huntington's disease, there was a significant decrease in putamen (control 5.33 ± 1.08, patient 2.68 ± 1.08), while in Alzheimer's disease, there was a marked loss of receptors in hippocampus (control 2.34 ± 0.62, patient 0.78 ± 0.61), in frontal cortex (control, 1.76 ± 0.19, patient 1.30 ± 0.22). Receptor density in temporal cortex showed a decrease, but did not achieve statistical significance (control 2.06 ± 0.21, patient 1.44 ± 0.64).

5 These data suggest a heterogeneous distribution of 5-HT$_4$ receptors in human brain, with high to moderate densities in basal ganglia and limbic structures. These receptors may not be principally co-localized on dopaminergic cell bodies or terminals, given the lack of change observed in Parkinson's disease. The loss of 5-HT$_4$ receptors in the putamen in Huntington's disease raises the possibility of their presence on intrinsic striatal GABAergic or cholinergic neurones. The marked loss of receptors in hippocampal and cortical regions in the brains from patients with Alzheimer's disease is consistent with a role for the 5-HT$_4$ receptor in cognitive processing.

Keywords: Human brain; 5-HT$_4$ receptors; Alzheimer's disease; Parkinson's disease; Huntington's disease; [^{3}H]-GR 113808

Introduction

The 5-hydroxytryptamine (5-HT)$_4$ receptor is a guanine nucleotide binding protein coupled receptor and has been cloned recently from rat. It has seven putative transmembrane spanning domains, and possesses a low homology (< 50%) to other 5-HT receptor subtypes (Gerald *et al.*, 1994). The receptor may exist as a long and a truncated form, each of which, when transfected, augments adenylyl cyclase activity (Gerald *et al.*, 1994). Activation of 5-HT$_4$ receptors in either rat oesophageal muscularis mucosae, mouse colliculi, guinea-pig hippocampus or human frontal cortex results in elevation of adenylyl cyclase activity (Ford *et al.*, 1992; Dumuis *et al.*, 1988; 1989; Eglen *et al.*, 1993; 1994; Monferini *et al.*, 1993). The 5-HT$_4$ receptor is potently stimulated by substituted benzamides or benzimidazalones, such as SC-53116 or BIMU-8 (Flynn *et al.*, 1992; Dumuis *et al.*, 1989), respectively, and antagonized, with subnanomolar affinity, by SB 204070, GR 113808 and GR 125487 (Wardle *et al.*, 1994; Grossman *et al.*, 1993; Gale *et al.*, 1994). [^{3}H]-GR 113808 (Grossman *et al.*, 1993), [^{125}I]-SB 207710 (an analogue of SB 204070; Brown *et al.*, 1993) or [^{3}H]-BIMU-1 (Jakeman *et al.*, 1994) have been used to characterize the pharmacology and distribution of 5-HT$_4$ receptors in mouse, rat, guinea-pig, porcine, bovine, macaque monkey and human brain (Waeber *et al.*, 1993; 1994; Domenech *et al.*, 1994; Jakeman *et al.*, 1994; Schiavi *et al.*, 1994).

The CNS distribution of the receptor is conserved across several species, although some minor differences are apparent between guinea-pig, mouse and rat in the globus pallidus, substantia nigra and interpeduncular nucleus (Waeber *et al.*, 1994). Furthermore, the receptor is found in macaque monkey, but not rat or guinea-pig lateral geniculate nucleus

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thalamus (Waeber *et al.*, 1994; Jakeman *et al.*, 1994). The heterogeneous brain distribution of the receptor implies a specific involvement in CNS function. The localization of the receptor in hippocampus, for example, suggests a role for the receptor in learning and memory (Waeber *et al.*, 1993; 1994; Domenech *et al.*, 1994; Jakeman *et al.*, 1994), while a high density in the nigrostriatal pathway suggests a role in extrapyramidal and motivational behaviour (Jakeman *et al.*, 1994; Domenech *et al.*, 1994). It is possible that regional pathophysiological changes in 5-HT_4 receptor density in neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease or Huntington's disease may elucidate their CNS function.

In the present study, the 5-HT_4 receptor in human *post mortem* brain tissue obtained from subjects with Alzheimer's, Huntington's and Parkinson's disease have been characterized. In addition, the receptor pharmacology and distribution in normal, age-matched, human brain have been described. The data obtained support previous reports (Waeber *et al.*, 1993; Domenech *et al.*, 1994) that demonstrate a high density of the receptor in human caudate nucleus and intermediate density in hippocampus. A preferential decline of receptor density in hippocampus was seen in patients with Alzheimer's, suggesting an association of the receptor with cholinergic, but not dopaminergic, systems.

Preliminary accounts of these data have been communicated to the British Pharmacological Society (Reynolds *et al.*, 1994a,b) and the third IUPHAR satellite meeting on Serotonin (Wong *et al.*, 1994).

Methods

Radioligand binding studies

Post mortem brain was homogenized in Tris HCl (25 mM) containing 0.5 mM EDTA at pH 7.4, followed by two cycles of centrifugation at 48,000 *g* and resuspension. Guinea-pig striatal homogenates were prepared according to the method of Francis & Burnham (1992). The final homogenate was incubated in Tris buffer at a 400 fold dilution (0.5 mg tissue/assay tube) with [^{3}H]-GR 113808 and competing agents, as appropriate, for 30 min at 21°C in triplicates. Non-specific binding was defined in either the presence of 10 μM tropisetron (ICS 205-930) or 100 μM 5-HT. The incubation was terminated by rapid filtration and washing with ice-cold buffer. The pharmacological specificity of the [^{3}H]-GR 113808-labelled binding sites were assessed by performing displacement studies with 0.05 nM radioligand and appropriate concentrations of inhibitors, using two concentration-ranges per decade. These experiments were performed on putamen samples from at least three different control subjects. In this tissue, the total counts per assay tube were typically between 1800–3000 d.p.m., 95% of which was specific displaceable binding. A single displacement experiment, with most compounds, was also carried out on frontal cortical tissue.

Regional distribution studies were performed by saturation analysis of specific [^{3}H]-GR 113808 binding at seven concentrations over a range of 6.25 pM–0.4 nM. At least three different control subjects were used for these studies. In studies on tissue from subjects with various neurodegenerative disorders, [^{3}H]-GR 113808 binding studies were undertaken at two ligand concentrations, 0.05 and 0.2 nM, due to the shortage of tissue for these studies. The higher concentration therefore related to the 5-HT_4 receptor density, while the ratio of binding at the two concentrations provided an estimate of ligand affinity, thereby controlling for artefactual effects of changes in apparent ligand affinity. The mean displaceable binding at 0.2 nM in control putamen was 89% of total.

Determination of the density of muscarinic receptors was performed according to the method of Nordberg *et al.* (1983). Tissues were homogenized in 80 mM sodium/potassium phosphate buffer at pH 7.4, followed by centrifugation at 48,000 *g* and resuspension. The final homogenate was resuspended in phosphate buffer solution at 500 fold dilution with [^{3}H]-quinuclidinyl benzylate (QNB) in a total volume of 0.25 ml for 60 min at 37°C, in triplicate. The incubation was terminated by rapid filtration through GF/B filters (pretreated in 0.05% polyethyleneimine) and washing with ice cold buffer. Non-specific binding was determined in the presence of 10 μM atropine. B_{max} values were determined by saturation analysis employing eight ligand binding concentrations between 0.02 and 2 nM.

Parkinson's, Alzheimer's and Huntington's diseases were confirmed in the patients by standard histopathological diagnostic criteria. Each series of tissue from patients with neurodegenerative disease was compared with tissues from an appropriate age-matched control group.

Analysis of data

Competition binding data were analysed by iterative curve fitting procedures from a four parameter logistic equation. The apparent affinity ($-\log K_i$) values of the competing ligands were calculated from IC_{50} values by the Cheng-Prusoff relationship (Cheng & Prusoff, 1973). Statistically significant differences were assessed by Student's *t* test.

Compounds used

BIMU-1 (endo-N-(8-methyl-8-azabicyclo[3.1.2]oct-3-yl)-2,3-dihydro-3-ethyl-2-oxo-1H-benzimidazole-1-carboxamide); BIMU-8 (endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-3-isopropyl-2-oxo-1H-benzimidazole-1-carboxamide); DAU 6215 (N-(endo-N-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxamide hydrochloride); DAU 6285 (endo-6-methoxy-8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2,3-dihydro-2-oxo-1H-benzimidazole-1-carboxylate hydrochloride); GR 113808 ([1-(2-methane-sulphonamido-ethyl)-piperidin-4-yl]-methyl-indole-3-carboxylate maleate), SDZ 205, 557 (2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino) ethyl ester), tropisetron (ICS 205,930) and (R)-zacopride (4-amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl) 5-chloro-2-methoxybenzamide hydrochloride), were synthesized in the Institute of Organic Chemistry, Syntex Discovery Research, Palo Alto, CA, U.S.A. All remaining compounds were obtained from Sigma Chemical Co., St Louis, MO, U.S.A. [^{3}H]-GR 113808 (87.9 Ci mmol^{-1}) was synthesized in the Institute of Organic Chemistry, Syntex Discovery Research, Palo Alto, CA, U.S.A. [^{3}H]-QNB (quinclidinyl benzylate; 60 Ci mmol^{-1}) was purchased from Dupont New England Nuclear, Boston, MA, U.S.A.

Results

Characterization of 5-HT_4 receptors in human brain tissue

The binding of the radioligand [^{3}H]-GR 113808 to various regions of human brain appeared to be saturable and of high affinity (Figure 1). The pharmacological nature of the site was characterized by using a series of displacing ligands (Table 1; Figure 2a,b). All compounds displaced bound ligand with a Hill coefficient not significantly different from unity. The rank order of apparent antagonist affinities was GR 113808> SDZ 205,557> DAU 6285> BIMU-1>DAU 6215> tropisetron> 5-HT> BIMU-8> (R)-zacopride. This rank order was similar to that observed in guinea-pig striatum (Table 1).

The distribution of 5-HT_4 receptors in human brain was heterogeneous (Table 2). Thus, high densities were seen in caudate nucleus, lateral pallidum and putamen. Low and

inconsistently detectable levels were seen in cerebellar cortex. Hippocampus, temporal cortex and amygdala expressed intermediate levels of 5-HT_4 receptor density. The apparent affinity of [^{3}H]-GR 113808 was similar throughout all regions studied and the saturation isotherms were consistent with an interaction at a single site (Table 2).

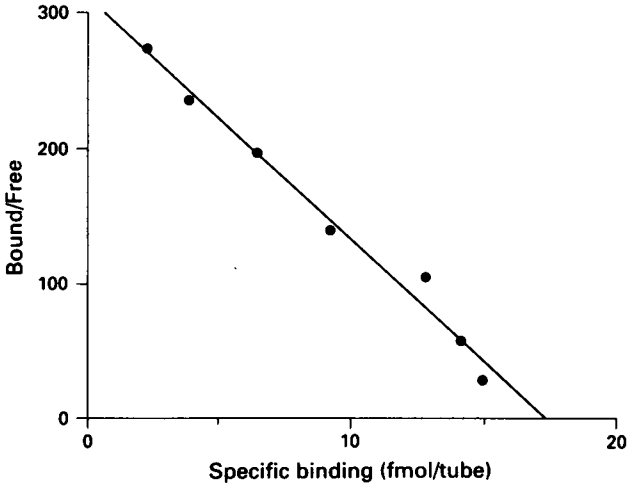

Figure 1 Representative Scatchard analysis of [^{3}H]-GR 113808 binding to membranes from human caudate nucleus. In this experiment the $-\log K_d$ value was 56 pM and the B_{max} was 7.0 fmol mg^{-1} tissue.

Table 1 Pharmacology of 5-HT_4 receptors in human *post mortem* brain tissue and guinea-pig striatum

Ligand	*Putamen*[a] $-\log K_i$	nH	*Cortex*[a] $-\log K_i$	*Striatum*[b] $-\log K_i$
GR 113808	10.05 ± 0.04	1.02 ± 0.12	10.12	10.20 ± 0.07
SDZ 205,557	8.65 ± 0.08	0.96 ± 0.20	8.32	8.63 ± 0.06
DAU 6285	7.95 ± 0.04	0.97 ± 0.06	8.20	7.55 ± 0.11
BIMU-1	7.81 ± 0.06	0.96 ± 0.09	7.75	7.91 ± 0.09
DAU 6215	7.42 ± 0.23	0.87 ± 0.25	7.23	ND
Tropisetron	7.39 ± 0.23	1.08 ± 0.03	7.38 ± 0.21	7.51 ± 0.02
5-HT	7.32 ± 1.00	0.95 ± 0.10	7.46 ± 0.04	7.52 ± 0.06
BIMU-8	7.25 ± 0.04	1.05 ± 0.05	ND	ND
(R)-zacopride	5.82 ± 0.04	1.01 ± 0.12	5.94	6.16 ± 0.02

Values are mean ± s.d., from three subjects, except for the single values obtained from human cortical tissue. The concentration of [^{3}H]-GR 113808 was 0.04–0.06 nM. The $-\log K_i$ values are calculated assuming K_d values of 73 pM for human putamen, 75 pM for human cortex and 30 pM for guinea-pig striatum.
[a]Human; [b]guinea-pig. ND – not determined.

Table 2 Regional density (B_{max}) and apparent affinity (K_d) of [^{3}H]-GR 113808 at 5-HT_4 receptors in human *post mortem* brain

Brain region	B_{max} (fmol mg^{-1} tissue)	K_d (pM)	n
Caudate nucleus	8.7 ± 1.5	68 ± 11	3
Lateral pallidum	8.6 ± 5.5	99 ± 30	8
Putamen	5.7 ± 3.0	94 ± 27	8
Medial pallidum	3.8 ± 0.9	90 ± 41	3
Temporal cortex	2.6 ± 0.6	69 ± 18	3
Hippocampus	2.4 ± 0.8	55 ± 25	3
Amygdala	2.3 ± 1.1	57 ± 25	3
Frontal cortex	1.7 ± 0.5	53 ± 23	3
Cerebellar cortex	< 1.0	ND	3

Values are expressed as mean ± s.d. from *n* subjects. ND, not determined.

Characterization of 5-HT_4 receptor density in neurodegenerative disorders

The levels of binding sites for [^{3}H]-GR 113808 in *post mortem* brains from patients with various degenerative disorders was also evaluated (Table 3). Most striking were decreases in binding of 67, 30 and 26% in hippocampus, temporal cortex and prefrontal cortex, respectively, in confirmed Alzheimer's disease brains. There was no significant deficit observed in the motor frontal cortex. No significant change in muscarinic receptor density, as determined by [^{3}H]-QNB binding, was observed in the same temporal cortex sample from Alzheimer patients reported above (control 56 ± 10; patient 58 ± 12 fmol mg^{-1} tissue).

No deficit in 5-HT_4 receptor binding was seen in putamen and substantia nigra from subjects with confirmed Parkinson's disease. However, a substantial deficit (approximately 50%) in 5-HT_4 receptor binding in putamen was observed in confirmed Huntington's disease patients.

Discussion

The availability of a high affinity and selective radioligand, such as [^{3}H]-GR 113808 (Grossman *et al.*, 1993), has allowed the distribution of 5-HT_4 receptors to be studied in several species. Waeber *et al.* (1993) and Domenech *et al.* (1994) have previously assessed, albeit from a limited number of tissue samples, the 5-HT_4 receptor distribution in human brain. The present study presents a more comprehensive characterization and assesses, for the first time, potential changes in 5-HT_4 receptors in three neurodegenerative disorders.

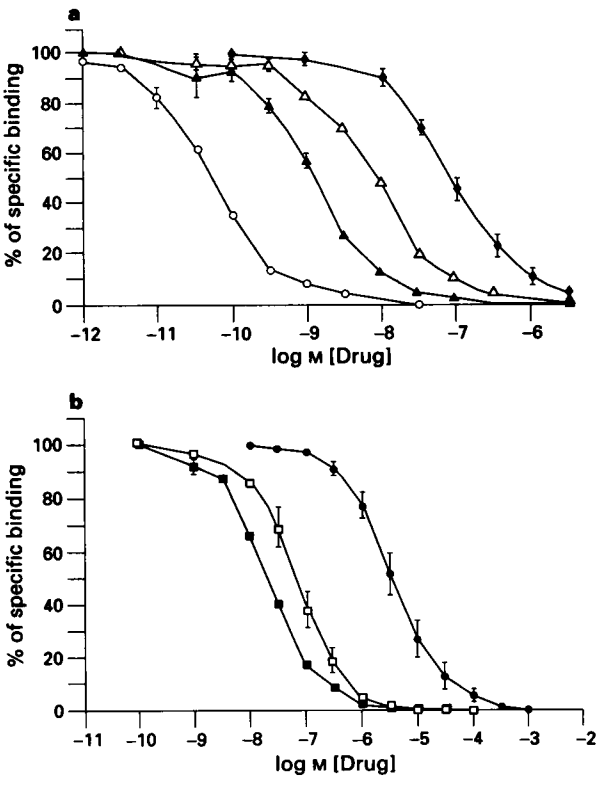

Figure 2 Competition displacement curves of [^{3}H]-GR 113808 binding in membranes from human putamen. The ligands were (a) GR 113808 (○), SDZ 205,557 (▲), BIMU-1 (△), 5-HT (◆) and (b) DAU 6285 (■), tropisetron (□) and (R)-zacopride (●). Values are mean with s.e.mean, from tissues removed, *post mortem*, from at least 3 patients. For abbreviations, see text.

Table 3 Levels of [^{3}H]-GR 113808 binding in *post mortem* brain tissue in various neurodegenerative diseases

Alzheimer's disease	*Binding* (fmol mg^{-1} tissue)	*Ratio*[a]	*Age* (yrs)	*Sex*	Post mortem *delay* (h)
Hippocampus					
Controls	2.34 ± 0.62	0.68 ± 0.25	75 ± 10	4F/4M	28 ± 11
Patients	0.78 ± 0.61***	0.64 ± 0.25	82 ± 8	6F/4M	33 ± 26
Temporal cortex (area 22)					
Controls	2.06 ± 0.21	0.71 ± 0.09	79 ± 5	3F/3M	26 ± 12
Patients	1.44 ± 0.64	0.66 ± 0.17	82 ± 10	3F/3M	25 ± 11
Frontal cortex (area 11)					
Controls	1.76 ± 0.19	0.71 ± 0.06	77 ± 6	3F/3M	23 ± 14
Patients	1.30 ± 0.22**	0.69 ± 0.06	81 ± 9	5F/3M	35 ± 27
Frontal cortex (area 4)					
Controls	1.43 ± 0.25	0.71 ± 0.06	84 ± 3	3F/3M	25 ± 9
Patients	1.18 ± 0.20	0.82 ± 0.10	82 ± 7	3F/2M	22 ± 4
Huntington's disease					
Putamen					
Controls	5.33 ± 1.08	0.60 ± 0.17	55 ± 9	1F/5M	39 ± 22
Patients	2.68 ± 1.08***	0.60 ± 0.06	57 ± 12	3F/3M	42 ± 23
Parkinson's disease					
Putamen					
Controls	4.74 ± 0.07	0.55 ± 0.15	64 ± 13	1F/5M	30 ± 25
Patients	5.86 ± 1.48	0.53 ± 0.10	69 ± 6	2F/4M	32 ± 33
Substantia nigra					
Controls	4.21 ± 2.56	0.57 ± 0.09	68 ± 7	1F/5M	31 ± 25
Patients	5.57 ± 0.10	0.62 ± 0.04	68 ± 6	2F/4M	32 ± 33

Values are mean ± s.d. of specific binding at 0.2 nM [^{3}H]-GR 113808 (fmol mg^{-1} tissue). [a]Ratio equals (specific binding at 0.05 nM)/(specific binding at 0.2 nM). **$P<0.01$, ***$P<0.001$ vs age-matched control group (Student's t test).

The rank order of ligand affinities in human putamen and cortex resembled rank orders observed at 5-HT$_4$ receptors in guinea-pig striatum (Table 1). Similar rank orders have been observed in caudate nucleus membranes from calf and pig (Grossman *et al.*, 1993; Domenech *et al.*, 1994; Schiavi *et al.*, 1994) suggesting limited species variation in the affinity profile of 5-HT$_4$ receptors. However, the absolute affinity values for the antagonists, GR 113808, SDZ 205,557 or DAU 6285, were somewhat higher than those obtained in observed functional studies (Ford & Clarke, 1993). Similar differences with tropisetron and SDZ 205,557 were seen in homogenate binding studies in guinea-pig striatum and hippocampus (Grossman *et al.*, 1993; Jakeman *et al.*, 1994), suggesting that species difference was not the cause. The reason for these discrepancies between functional and binding studies therefore remains unclear. In the case of differences in the apparent affinity of GR 113808, the temperature of the binding assay is an important factor (Waeber *et al.*, 1993; 1994; Wong *et al.*, 1995).

The data obtained in the present study demonstrate a heterogeneous distribution of 5-HT$_4$ receptors in human brain, with the highest densities of receptors in caudate nucleus, pallidum and putamen and intermediate levels in cortex, hippocampus and amygdala. These findings are consistent with those reported in human, rat and primate brain (see Introduction for references). The discrete localization of 5-HT$_4$ receptors, as labelled by [^{3}H]-GR 113808, implies specific CNS functions. In particular, the association of the receptor with septal-hippocampal or nigra-striatal pathways, suggest an involvement in cognitive, emotional and motor function control.

The density of 5-HT$_4$ receptors in regions of human brain, even in caudate nucleus, was low in comparison to the density of 5-HT$_4$ receptors in non-human brain. The effects of agonal state, age or other factors associated with *post mortem* tissue collection and storage may contribute to these low densities. Alternatively, species differences in CNS distribution of 5-HT$_4$ receptors is the most probable reason. For example, in man and guinea-pig, 5-HT$_4$ receptors are localized to the pars reticula of substantia nigra (Waeber *et al.*, 1994), whereas in rat and mouse, they are found only in the pars lateralis. The density of 5-HT$_4$ receptors was also low in comparison to the density of 5-HT$_2$ receptors, although not as low as the density of 5-HT$_3$ receptors (Barnes *et al.*, 1989; Domenech *et al.*, 1994). It is possible that a low density of 5-HT$_4$ receptors reflects a modulatory role of 5-HT$_3$ or 5-HT$_4$ receptors within the CNS (Costall & Naylor, 1994), in comparison to a dominant excitatory or inhibitory role of amino acid or cholinoceptors (Iversen, 1984), that are present within the CNS in higher densities. However, *in lieu* of data regarding the neuronal localization of 5-HT$_4$ receptors, the precise reason for the low density remains speculative.

Insight into 5-HT$_4$ function in these areas can be gained by studying changes in 5-HT$_4$ receptor density in various degenerative disorders. In the present study three such diseases were evaluated, two of which, Huntington's disease and Parkinson's disease, are associated with changes in the nigrostriatal pathway, and a third, Alzheimer's disease, is associated with changes in hippocampal and cortical function. The most striking changes in the present study were seen in the selective loss of hippocampal 5-HT$_4$ receptors in Alzheimer's brain. This reduction was greater than reported for 5-HT$_{1A}$ or 5-HT$_3$ receptors in the same region (Cross *et al.*, 1984; Barnes *et al.*, 1990), although the decrease was similar to that reported for 5-HT$_2$ receptors (see Greenamyre & Maragos, 1993, for review).

Case histories of patients in the present studies indicated that there was no record of the administration of any drug likely to compete for the 5-HT$_4$ receptor. In addition, the absence of any increase in the binding ratio (Table 3) indicated that no significant difference in apparent receptor affinity occurred between neurodegenerative disease and control cases. Therefore, competition of such drugs for the receptor was unlikely to be responsible for the diminished binding in Alzheimer's and other disease tissues. A plausible reason for the reduction in 5-HT$_4$ receptors in Alzheimer's brain is the loss of neurones on which they are expressed. It

is unlikely that these changes reflect a general degeneration of the brain occurring from *post mortem* ischaemia or oxidative insult, since in the temporal cortical area, there was no corresponding change in muscarinic receptors. Moreover, no significant deficit is found in hippocampal [^{3}H]-QNB binding sites in Alzheimer's disease (Nordberg *et al.*, 1983). It is presently unknown upon which neurones the 5-HT$_4$ receptors are located. However, they may reside on cholinergic neurones, given their association with the septal-hippocampal pathway and the well-documented cholinergic deficits in Alzheimer's disease (Katzman, 1989). It is possible, therefore, that the loss of 5-HT$_4$ receptors may provide a marker for degenerating cholinergic terminals, as also suggested for the 5-HT$_2$ receptor (Greenarmyre & Maragos, 1993).

The location of 5-HT$_4$ receptors in hippocampus from several species, including man (see Introduction for references), is also consistent with a role for the receptor in learning and memory (Bockaert *et al.*, 1994a). In support of this hypothesis, several lines of evidence have been reported. Thus, Rowchoudhury & Anderson (1995) have presented preliminary data indicating that activation of 5-HT$_4$ receptors facilitates long term potentiation (LTP) in the CA1 region of rat hippocampus. In mouse colliculi neurones, moreover, 5-HT$_4$ receptor agonism elevates intracellular adenylyl cyclase and, consequently, inhibits voltage-sensitive potassium channels opening time (Dumuis *et al.*, 1989; Andrade & Chaput, 1992; Bockaert *et al.*, 1994a,b). Prolonged closure of potassium channels, and thus neuronal hyperexcitability, was seen, even after very short exposures to 5-HT (Bockaert *et al.*, 1994b). These mechanisms may be involved in the induction of hippocampal CA1 late stage LTP (Frey *et al.*, 1993); a potential mechanism for explicit forms of memory. Finally, RS 66331-190, BIMU-1 or BIMU-8, (potent, but mixed 5-HT$_4$ agonists/5-HT$_3$ antagonists), enhance cognitive performance in rodents (Fontana *et al.*, 1994; Ghelardini *et al.*, 1994).

Huntington's disease is a condition associated with a profound neuronal loss in the basal ganglia, such as the caudate nucleus, putamen and pallidum (Reynolds *et al.*, 1990). In Huntington's disease, there is a decrease in glutamate and GABA levels in the putamen, which may be related to the dementia associated with late stages of the disease (Reynolds *et al.*, 1990). The data obtained in the present study indicate a decrease in 5-HT$_4$ receptors in the putamen, suggesting that these receptors may also be associated with either glutamatergic transmission or intrinsic GABAergic neurones. This suggestion is supported by ontogenic studies of Waeber *et al.* (1994), showing a synchronous, albeit transient, expression of 5-HT$_4$ receptors and glutamatergic innervation in rat globus pallidus. The decrease in putamen 5-HT$_4$ receptors binding may alternatively, or additionally, be due to the presence of receptors on cholinergic interneurones, which also decline in the disease (Forno, 1992; Greenamyre & Maragos, 1993). It is at present unclear, however, whether the reduction in 5-HT$_4$ receptors plays a role in the progression of dementia and/or the appearance of dyskinesias.

The lack of change in the density of binding to 5-HT$_4$ receptors in the basal ganglia areas in patients with confirmed Parkinson's disease indicates that the receptor is not principally expressed on dopaminergic cell bodies or terminals in the substantia nigra or putamen, even though the 5-HT$_4$ receptor is present in high density. Indeed, 6-hydroxydopamine lesions in rat striatum (Jakeman & Fontana, 1994 unpublished observations; Dumuis *et al.*, 1994) further support an absence of the 5-HT$_4$ receptors on dopaminergic neurones.

In conclusion, the distribution and characterization of 5-HT$_4$ receptors in human brain, is well conserved across species. The existence of the receptor in the nigra-striatal pathway is intriguing given the lack of change seen in Parkinson's disease. These data may suggest a dissociation between the 5-HT$_4$ receptor and the dopaminergic system, while the data from Huntington's disease suggest the presence of the receptor on intrinsic striatal GABAergic or cholinergic neurones. The location of the receptor in hippocampus and its decline in terminal Alzheimer's disease highlights a possible association with the cholinergic system and supports the involvement of 5-HT$_4$ receptors in cognition.

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